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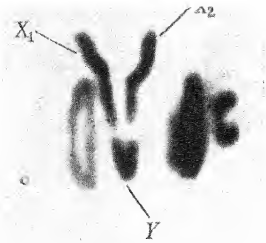

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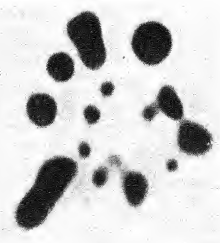

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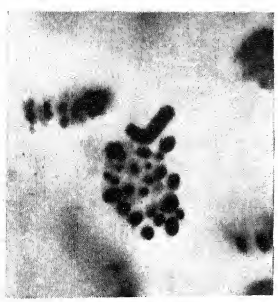

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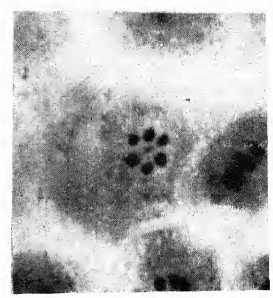

FIG. 4.

FIG. 1.—The X_1X_2Y sex-trivalent in the Mantid *Sphodromantis viridis*. Metaphase of first meiotic division (side view).

FIG. 2.—Metaphase of first meiotic division in the long-horned grasshopper *Pholidoptera griseoaptera* (16 bivalents, X-chromosome not visible).

FIG. 3.—Spermatogonial metaphase in the long-horned grasshopper *Leptophyes punctatissima* (31 chromosomes, of which the X is the largest).

FIG. 4.—Second meiotic division in the Coreid Heteropteran *Archimerus calcarator* (7 autosomes, the smallest in the centre).

THE CHROMOSOMES

by

M. J. D. WHITE, D.Sc.
LECTURER IN ZOOLOGY, UNIVERSITY COLLEGE, LONDON

WITH A FRONTISPIECE
AND 21 OTHER ILLUSTRATIONS

SECOND EDITION, REVISED

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PREFACE

I HOPE that this book will be of use to many biologists who realize that chromosome-cytology has made considerable progress in the last ten years, and that many of the elementary accounts of mitosis and meiosis are hopelessly inaccurate, but who have no time to read the larger works of Wilson, Darlington and Bělař, which must remain the standard sources of information on the subject.

Chromosome cytology is essentially a practical subject, which can only be thoroughly mastered by a study of actual preparations under the microscope. Unfortunately this study is usually regarded as too difficult to be included in a degree course in biology. It is surprising, however, how much can be seen, even without using an oil-immersion objective, provided that one chooses suitable material with large chromosomes. There is no doubt that for most purposes the testes of Locusts and Grasshoppers (any species will do) provide the best introductory material. They should be fixed in San Felice's fixative (1 per cent chromic acid 16 parts, Formalin 8 parts, glacial acetic acid 1 part—freshly mixed just before use) or in Flemming's solution and stained in one of the aniline dyes like Gentian Violet. In the course of the past few years I have made ordinary degree students work through material of this kind (sectioned at 20–25 μ so as to obtain whole nuclei). They were able to see all the stages of mitosis and meiosis and even to work out the average number of chiasmata per nucleus in several different species. That it is possible for students to do this in a course involving only one afternoon a week should destroy the myth that cytology is a particularly difficult subject. Preparations of salivary gland chromosomes

can easily be made from larvae of many species of Diptera. *Drosophila* and *Chironomus* larvae are particularly convenient, but blowfly and mosquito larvae are unsuitable. The procedure is to dissect out the glands, and stain them for a few minutes in a saturated solution of carmine in 45 per cent acetic acid (a 1 per cent solution of Orcein in 45 per cent acetic acid is probably better). After staining in a drop of the fluid they should be transferred to clean 45 per cent acetic acid and crushed under a cover-glass (the best method is to roll a glass rod several times over the cover-glass—only experience can teach the exact amount of pressure needed to ensure crushing). The preparation is then ready for study, but may be sealed with vaseline or paraffin in order to improve its keeping qualities (such preparations will often last for several weeks).

In a book of this size it is necessarily not possible to quote ' chapter and verse ' for each statement. I mention this in apology for a certain amount of dogmatism imposed by limitations of space. The illustrations are essentially diagrammatic and designed to illustrate principles rather than to serve as actual illustrations of cell-division in particular organisms.

In any subject it is impossible to avoid technical terms, but I have endeavoured to reduce them to a minimum, and have explained in the text all those which are not self-explanatory. Where a term occurs for the first time and is defined it is printed in italics. In the description of meiosis I have adopted the term *bivalent* instead of *tetrad* as being far less likely to cause confusion.

For those who find certain parts of the book difficult to understand, I would recommend the use of some models, which can be constructed in a few minutes out of soft copper wire or plasticine, using the illustrations as a guide. With the aid of these it should be possible for anyone to understand the details of chiasma-formation and meiosis.

PREFACE TO THE SECOND EDITION

IN the five years which have elapsed since the first edition of this book was written many important advances have been made in our knowledge of chromosome-structure, chromosome-function and the principles which govern the evolutionary transformations of chromosomes. A short section on the chemical composition of the chromosomes has been added to Chapter II, and Chapter VI (Chromosomes and Evolution) has been entirely re-written. A number of other changes have been made in order to include recent discoveries of importance. The term *centromere* has been adopted in preference to *spindle attachment*, since it now seems to have gained general (although not universal) acceptance. The frontispiece has been added in order to illustrate various points and to give beginners some idea of what good chromosome preparations should look like under the microscope.

M. J. D. W.

August, 1942

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CHAPTER I

THE RESTING NUCLEUS

THE term 'resting nucleus' is unfortunate, since it seems to imply that the metabolic activities of the nucleus are reduced to a minimum when it is not dividing—a view for which there is no evidence. The alternative term 'metabolic nucleus' is equally unfortunate in that it suggests that dividing nuclei are physiologically inactive. On the whole it seems best to retain the established, although misleading, term.

The *resting nucleus* is, then, one which is not dividing. Usually it remains optically unaltered for long periods—it is not obviously changing either its shape or its appearance. This is, however, not always so; many resting nuclei increase steadily in size (either by uptake of water or by actual 'growth') and may alter their shape or appearance in various ways without dividing.

The chief structural parts of the resting nucleus are the *nuclear membrane*, the *nuclear sap* and the *chromosomes*; the bodies known as *nucleoli* are attached to certain chromosomes (always the same ones, for a particular organism) and are hence to be looked upon as parts of those chromosomes, rather than as independent constituents of the nucleus.

The nuclear membrane has been shown by microdissection to be a definite structure with physical properties. If a fine needle is gently pushed against it, it becomes indented at the point where the pressure is applied; if the pressure is released it regains its former shape; if the pressure is increased it can eventually be punctured.[23] The shape of the nucleus undoubtedly depends in part on the properties of

the nuclear membrane. Most nuclei are approximately spherical, but many are ovoid. Those of many Vertebrate leucocytes are in the form of a long strand with periodic enlargements (Fig. 1*a*) while those of the secretory cells of many insects are very irregularly branched (Fig. 1*b*).[173] In all these cases of non-spherical nuclei the nuclear surface is very large relative to its volume, and it has been assumed that this is connected with the process of secretion; but many secretory cells (such as the salivary gland cells of Diptera) have approximately spherical nuclei. Some unusual nuclei do not consist of a single body at all, but of a number of separate vesicles, each containing one or more chromosomes and a certain amount of nuclear sap inside a separate membrane (Fig. 1*c*). In some cases the sex chromosome is enclosed in a separate membrane from the main nucleus (Fig. 1*d*).

The nuclear sap is usually a clear fluid; its viscosity has been determined in one case to be about twice that of the water [57], and this is probably typical of most nuclei. The amount of nuclear sap relative to the volume of the chromosomes varies enormously from one type of nucleus to another. Thus in the micronucleus of Ciliates and in the spermhead nuclei of many animals there is practically no nuclear sap; on the other hand, the total volume of the young oocyte nuclei of birds (diameter up to 100 μ) may be 200,000 times that of the chromosomes at metaphase.

The chromosomes may be, and usually are, invisible in living nuclei during the resting stage.[60 100] In some plant nuclei, however, and also in a few animal nuclei, fine threads can be seen in the living resting nucleus, which are almost undoubtedly chromosomes, although highly hydrated and almost invisible, due to their having nearly the same refractive index as the nuclear sap. That the chromosomes do actually persist through the resting stage is certain, since in some cases they become visible at the beginning of one

mitosis in the same position as they occupied at the end of the preceding division.[13] Also, in some cases

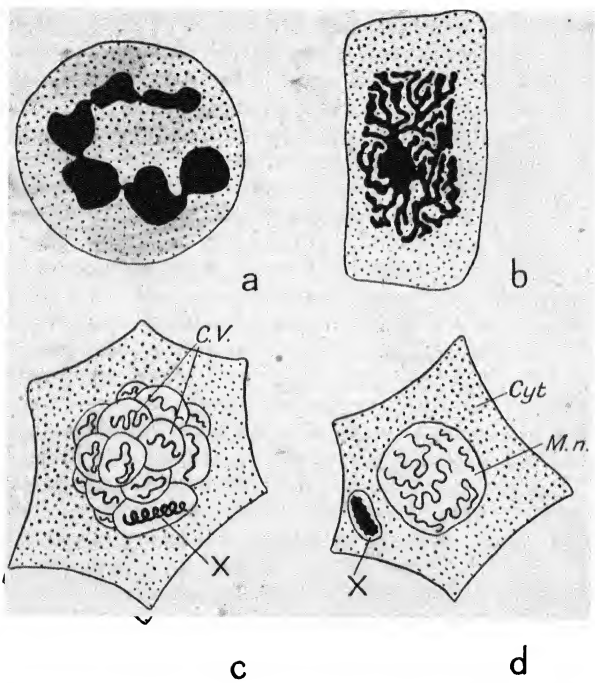

FIG. 1.—Unusual types of nuclei; *a* in a vertebrate marrow-cell; *b* in one of the spinning gland cells of *Platyphylax* (Caddis-fly); *c* in a spermatogonium of the grasshopper *Aularches*; *d* in a spermatogonium of the Bush-Cricket *Pholidoptera griseoaptera*. In *c* each chromosome lies in a separate nuclear membrane, in *d* only the sex chromosome lies in a separate membrane. Cyt. = cytoplasm; C.v. = chromosomes lying in nuclear vesicles; X = X-chromosome.

special portions of chromosomes persist in a condensed state throughout the resting stage (*prochromosomes*).

It is now becoming increasingly clear that no idea

of the structure of the resting nucleus can be obtained from studying fixed preparations. The usual text-book figure of a 'network of linin threads with granules of chromatin at the points of intersection' is meaningless save as a description of a gross artefact which bears only the most remote relation to the living structure. We must resign ourselves to the fact that the resting nucleus and its chromosomes, due probably to their high water content, are *unfixable*. It should be emphasized that this only applies to resting nuclei—there is every reason to believe that fixed preparations of nuclei in mitosis present a very accurate picture of what is taking place in the living cell (see next chapter).

Most cells in the body of an adult human being have undergone about 50 mitoses since the fertilized egg (i.e. an adult man consists of about 10^{14} cells, making allowance for the erythrocytes, sperms and other cells which are constantly being destroyed and replaced). In the case of insects the adult cells have undergone only about 20–30 divisions, and in the case of the Nematoda and Rotifera still fewer. Between each of these divisions a resting stage has intervened. These resting stages are, however, of very uneven duration; on the one hand two divisions may follow on one another without being separated by any resting stage at all (the end of one division passing directly into the beginning of the next)—or on the other hand the resting stage may last for years as in many adult tissues of vertebrates. Most adult cells may be said to have entered a permanent resting stage, since they will never divide again. Usually the resting stage lasts a much longer time than the few hours required to undergo mitosis, but this is not always so. Some nuclei begin to undergo division and then become arrested, remaining in a particular stage of mitosis for the greater part of their life-cycle. Others may be caused to do so by specific chemical agents such as auramine, sodium cacodylate and

colchicine.[102] Moreover in Vertebrate oocytes with much yolk (Sharks, Amphibia and Birds) the chromosomes may remain in one stage of meiosis for many months.[25, 154]

This naturally leads to a consideration of the question: what is it which causes a nucleus to leave the resting stage and enter on the complicated system of changes which we call mitosis? In certain tissues such as cleaving embryos and frequently in lobules of the testis, division takes place synchronously in all the cells—that is to say every nucleus will be in exactly the same stage at any given moment. On the other hand in epithelia such as the skin and the gut-lining, isolated cells enter on mitosis quite sporadically and independently of the neighbouring cells. In the first case we appear to have a 'tissue-control' of mitosis and in the second a 'cellular control'. Probably in synchronously dividing tissues the substance which is inducing mitosis can diffuse freely from cell to cell (perhaps as the result of protoplasmic 'bridges' between the cells), while in the second case it is unable to do so.

It must not be assumed that there is a single mitosis-producing agent. Possibly there is, but if so it is as yet undiscovered. But it is certain that a large number of physiological conditions are capable of stimulating cell division and it seems probable that in natural tissues more than one agency may be effective. In Fishes and Amphibia prolonged starvation followed by a meal may cause a considerable increase in the number of dividing nuclei, while in other cases starvation alone may be sufficient.[129] Such diverse stimulants as a peritoneal injection of foreign blood serum [48] or even a bacillus culture [75] have been found to produce the same effect. What the underlying chemical mechanism is in these cases we have no idea. It must be pointed out that a mere increase in the number of dividing nuclei in a histological section is not sufficient to prove that mitosis

has been stimulated in resting nuclei ; it may equally well result from a slowing down of mitosis.

Gross mechanical injury to the nucleus such as results from the withdrawal of a microdissection needle previously inserted into the nucleus, will often produce a sudden acceleration of mitosis [23] but in some cases it will cause the normal course of mitosis to be reversed, so that nuclei which have already entered on the division cycle go back into the resting stage.[174]

The effects of irradiation with X-rays also vary from one type of tissue to another. In some cases mitosis ceases altogether in a tissue for some days after irradiation ; cells which would have entered on mitosis are retarded in the resting stage.[117] In other cases X-rays probably accelerate the onset of mitosis. The whole subject of nuclear pathology is urgently in need of re-interpretation. Thus many nuclei when injured or dying become '*pycnotic*', that is to say their chromosomes become fused into a single large mass which stains intensely with dyes like Haematoxylin and anilin derivatives. Pycnosis is probably to be interpreted as a highly modified non-functional mitosis, since in some cases [147] pycnotic nuclei may begin to divide, but we do not know what actually happens to the chromosomes in a pycnotic nucleus.

Some recent authors have omitted the primary distinction between two forms of nuclear division, *mitosis* and *amitosis*, regarding all kinds of amitosis as merely modified or concealed mitosis. While it is clear that most of the nuclear phenomena in Protozoa which were formerly regarded as amitosis are best considered in this light, there is no doubt that true amitosis occurs in many somatic tissues of insects (fat body, genital ducts, &c.). Here the chromosomes probably divide and separate into their halves in a 'resting' nucleus and are then passively distributed in approximately, but not exactly, equal

numbers to the two daughter nuclei into which the original nucleus divides by elongation into a 'dumb-bell' shape.[114] In some somatic nuclei the chromosomes divide repeatedly within the nuclear membrane without any true mitosis—thus doubling the chromosome number again and again. This phenomenon ('endomitosis') has been particularly studied in insects [54], but it probably occurs in other groups as well.

CHAPTER II

THE GENERAL OUTLINE OF MITOSIS

WE have seen in the previous chapter that the stimuli which can cause the onset of mitosis are extremely diverse; they may be regarded as external agencies releasing an inherent chain of biochemical and biophysical events in the nucleus. If the cell is almost ready to divide in any case, stimulation by a mitogenetic agent or puncturing the nuclear membrane will produce a more or less normal mitosis; if the mitotic mechanism is not 'wound up' then pycnosis results. A study of the gross disturbances which lead to mitosis does not help us much to understand the internal causes normally responsible for the initiation of the whole cycle of events; neither does it explain how in some cases that cycle may be interrupted at certain stages and then subsequently resumed.

It is usual to divide mitosis into four stages, *prophase*, *metaphase*, *anaphase* and *telophase*. For convenience in description it seems best to subdivide anaphase into two parts and to insert a stage which can be called *prometaphase* between prophase and metaphase.

1. Prophase

At the beginning of prophase the chromosomes become 'fixable'—that is to say, their appearance in fixed material approximates closely to that seen in living cells by the most reliable methods of observation. This 'fixability' increases throughout prophase until at prometaphase and metaphase there is every reason to believe that fixed and stained preparations give us an almost perfect picture of the appearance in the living state. In the majority of nuclei (all those which have not got prochromosomes) the fixability is zero during the resting stage—thus the first sign of prophase is the appearance of visible threads (chromosomes) in the nucleus in place of the network which results from the fixation of a resting nucleus.

The 'fixability' of early prophase nuclei varies a good deal, but appears to be correlated with the volume of the nuclear sap in which the chromosomes lie; thus in the first spermatogonial division of the grasshopper *Metrioptera* the nucleus is large and the prophase chromosomes very 'fixable'; in the succeeding divisions the nucleus gets progressively smaller and the chromosomes less fixable until the sixth division when there is a sudden increase in the size of the nucleus which is accompanied by an increase in fixability; the seventh and eighth divisions again show small nuclei in which the early prophase chromosomes fix badly.

The question now arises: what is the physical basis of this property of fixability? There is considerable evidence that it depends on the degree of colloidal hydration of the chromosomes. Bělař[7] showed by experiments on the dehydrating power of hypertonic solutions on the nucleus that the metaphase chromosomes contain less water than any other nuclear constituent and we have reason to believe

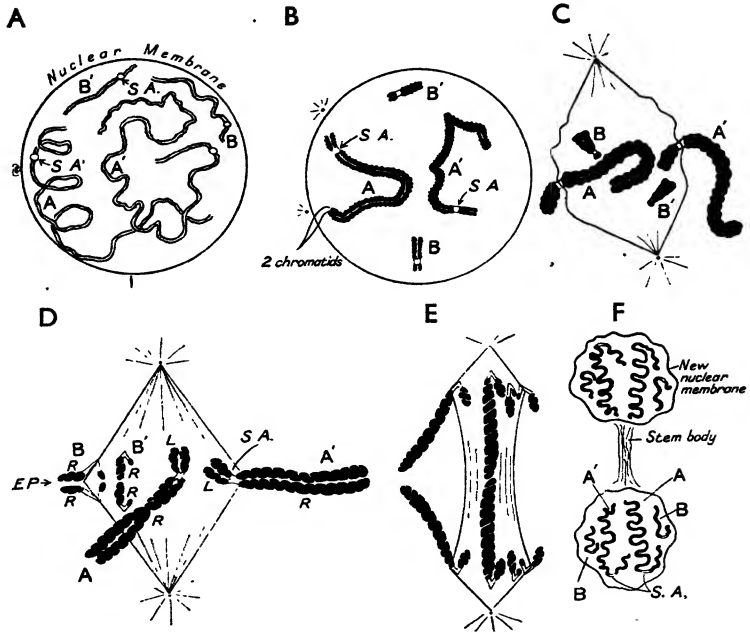

FIG. 2.—Diagrams of the main stages of mitosis. Only two pairs of chromosomes A and A′, B and B′ are shown. Both of these have sub-terminal centromeres, those of the B chromosomes being nearer the end. At early prophase the 'relic spirals' are clearly seen. S.A.=centromeres, E.P.= equatorial plane of the spindle. R and L are regions of the chromosomes which are spiralized in a right or left-handed direction at metaphase.

that the resting-stage chromosomes are highly hydrated, so one must infer that dehydration is one of the processes involved in prophase.

In all cases the chromosomes at the very earliest prophase are separate; there is thus no 'continuous spireme' as described by some of the earlier cytologists. Further, the individual chromosomes are always double from the very beginning of prophase, with the two threads or *chromatids* of which they are composed closely approximated throughout their length. Where there are size differences between the chromosomes the ratio of the lengths at early prophase is approximately equal to the ratio of the volumes at metaphase and it is possible to pick out the pairs of chromosomes in a diploid organism.

The appearance of early prophase chromosomes depends to a certain extent on whether the preceding resting stage has been of long or short duration. Where it has been short and the chromosomes are few in number as in the cleavage divisions of the Horse Roundworm, *Ascaris megalocephala*, it is possible to show [13] that the individual chromosomes become visible in the same positions as they had disappeared in at the previous telophase. Again, where the resting stage has been short and the chromosomes are relatively long and 'fixable' it can be seen that at the early prophase stages they are coiled into loose spirals (Fig. 2*a*). The two chromatids are always closely approximated from one end of the spiral to the other.

As prophase proceeds the volume of the chromosomes increases considerably; there is thus an actual manufacture of new material during prophase. Side by side with this a shortening and thickening of the chromatids takes place. We have thus identified three processes which are involved in the development of prophase: dehydration, growth, and condensation or contraction; we must now add a fourth—'despiralization'. That is to say that as

shortening and thickening take place the spirals of the early stages unwind.[34]

Chemical and especially spectroscopic methods have shown that the permanent framework of the chromosome is composed of protein and that the substance which accumulates during the thickening of the chromosomes is nucleic acid, a compound formed by the polymerization of nucleotides. Each nucleotide is formed by the union of a phosphoric acid molecule, a molecule of a pentose-sugar and a purine or pyrimidine group. Single isolated nucleotides occur in the cytoplasm, and are present in large quantities in the cytoplasm of cells which are about to undergo a series of mitoses.[19] The nucleic acid of the chromosomes is clearly derived from the cytoplasmic nucleotides which are eventually built up into long fibre-like molecules, each containing about 2,000 nucleotides. It is probable that the nucleic acid and protein parts of the chromosome are chemically combined to form salt-like nucleoproteins. Similar nucleoproteins are found in many enzymatic systems, and virus particles are also made of the same type of compound, so that it is probably the essential basis for the continued building up of protein molecules in living systems. There is an interesting difference between the cytoplasmic nucleotides and the nucleic acid of the chromosomes : whereas the pentose of the former is d-ribose, in the latter the carbohydrate constituent is a desoxy-sugar. Thus a chemical change takes place in the structure of the nucleotides at the time of polymerization.[189]

The Feulgen-reaction (staining with fuchsin-sulphurous acid after a preliminary hydrolysis) has generally been regarded as specific for ' chromatin '— actually it depends on the presence of the desoxy-sugar and is hence not given by the ribose-nucleotides of the cytoplasm.

In well-fixed chromosomes it is possible to see from the very beginning of prophase that the staining substance of the chromatids is not continuous from

end to end; it is interrupted at one point at least (in plant chromosomes usually several points) to form a non-staining gap (Fig. 2). These gaps become more obvious later, and are called *constrictions*: their position is constant for each chromosome. They are filled by a non-staining substance which is not nuclear sap and which holds the chromatids on either side of it together.

Throughout the prophase of mitosis the outlines of the chromatids present a slightly irregular woolly or hairy appearance which is probably an artefact: they do not in general show a series of granules (*chromomeres*) such as are seen at the meiotic prophase; this may be a real difference and not due to difference in fixability. By the end of prophase the woolly appearance referred to above has almost disappeared and a smooth outline has taken its place.

The long threads of the early prophase chromosomes appear to wind more or less at random throughout the nuclear cavity; but they never actually come in contact with one another, or indeed approach within a certain minimum distance: there is thus something which keeps them apart, which is probably in the nature of a generalized electrostatic repulsion distributed over the surface of the chromosome. As prophase advances there is a tendency for the shortened and thickened chromosomes to move to the periphery of the nucleus and to arrange themselves on the inner surface of the nuclear membrane.

If a nucleolus or nucleoli are present in the resting stage they usually lose their staining power during prophase and have disappeared completely by prometaphase. This is not the case, however, in many of the Protozoa, where what appear to be nucleoli often persist through the entire mitotic cycle.[4] It is now known that the nucleoli are always connected to particular chromosomal regions which may be regarded as *nucleolar* organizers. It seems best to look upon nucleoli as reservoirs of nucleotides which are

converted into nucleic acid during mitosis and utilized in the nucleination of the body of the chromosome. In *Drosophila melanogaster* the nucleolar organizers lie in the X and Y chromosomes ; in Maize they lie in the VIth chromosome.[105]

2. Prometaphase

At the end of prophase the nuclear membrane usually disappears. In many of the Protozoa, and even in some higher forms, however, it persists and the whole process of mitosis is intranuclear.[4] The term prometaphase designates the period from the dissolution of the nuclear membrane up to the end of the process of spindle-formation. In 'intranuclear mitosis' there is no stage which can be separately distinguished as prometaphase.

The mode of origin of the spindle varies considerably, but it is probably possible to reduce the essential details to a common plan. In the simplest cases it is formed (probably entirely out of nuclear sap after the dissolution of the membrane) as separate spindle-elements, corresponding in number to the chromosomes. This is the type of spindle found in the meiosis of some scale-insects[70] and in the female meiotic divisions of *Artemia salina*, the Brine Shrimp (Fig. 12).[59] Usually these spindle elements fuse completely to form a single gelatinous body in which the separate elements are no longer visible (Fig. 2*c*, *d*), but in the above cases they remain distinct, and do not even converge towards 'poles' but end in fan-shaped expansions (Fig. 3*a*). In these and a number of other cases no trace can be found of centrosomes or asters, which leads one to the conclusion that, whatever their relation to the spindle when present, they are at any rate not essential to the division process. Where centrosomes and asters are present (as in the majority, but by no means all the Metazoa) an apparent spindle may form between them outside the nuclear

membrane. In this case when the membrane disappears this structure (which is a good deal smaller than the final spindle and can be called the *central spindle*) moves into the middle of the nuclear area. The nuclear sap then apparently undergoes rapid gelation round the original central spindle so as to increase its volume considerably. Thus a compound spindle is formed which differs from the previous types only in having a central element not formed of nuclear sap but of extranuclear cytoplasm.[6]

The development of this central part of the spindle outside the nucleus in some cases has proved very confusing, since it has led to the conception of the chromosomes attaching themselves to a preformed spindle; actually they are associated with the true spindle elements as soon as the latter are formed, and are probably never attached to the central element.

3. Metaphase

At the end of prometaphase (the period in which the spindle is formed) the chromosomes are 'attached'—the term is misleading, but has to be retained—to the spindle in the region of the equator, that is to say equidistant from its two ends. The arrangement of the chromosomes at metaphase depends on a number of factors, (1) whether a central spindle element is present or not, (2) the number of the chromosomes, and (3) their sizes. Where the chromosomes are very long or a large central element exists, as in the dividing leucocytes of Salamandra [6] all the chromosomes are arranged round the periphery of the equator, irrespective of their number; their points of attachment are approximately equally spaced round the edge, so that if there are six chromosomes they will be 60° apart, if there are 24 (as in Salamandra) they will be 15° apart. Here it appears that the spindle elements associated with the chromosomes form a circle round

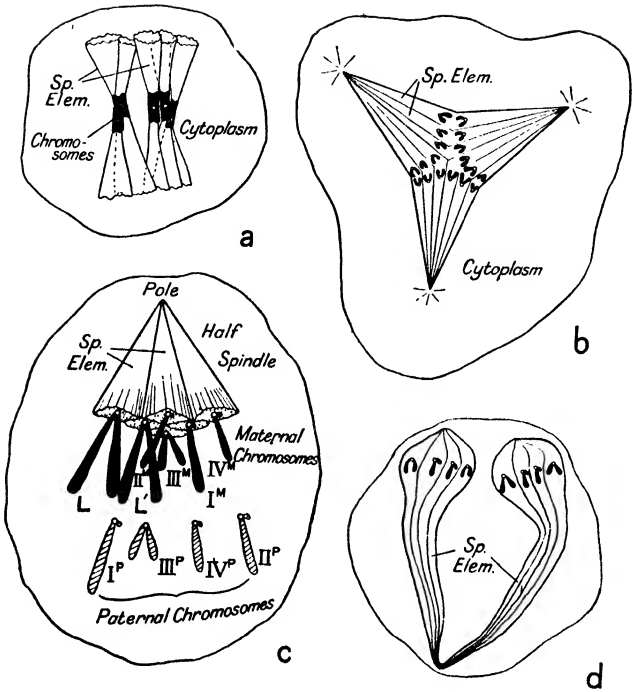

FIG. 3.—Various unusual types of spindles. *a* = the spindle at the first meiotic division in the scale insect *Llaveia bouvari* [70] where the spindle elements are quite separate. *b* = a tripolar spindle at early anaphase. *c* = the half-spindle formed at the first meiotic division in the fly *Sciara coprophila* [124]. *d* = a late anaphase spindle in a hybrid where the central region has undergone great elongation[41]. In *c* all the maternal chromosomes (including the two 'limited' ones L and L') go to the pole, the paternal ones (I^P, II^P, III^P and IV^P in order of size) moving in the opposite direction.

the central element (Fig. 4*a*). In some organisms the two chromatids at this stage are strictly parallel, in others they wind round one another (cf. Fig. 5*b* and *i*).

Where there is no central element some of the chromosomes may be entirely embedded in the middle of the spindle.

These arrangements are found when all the

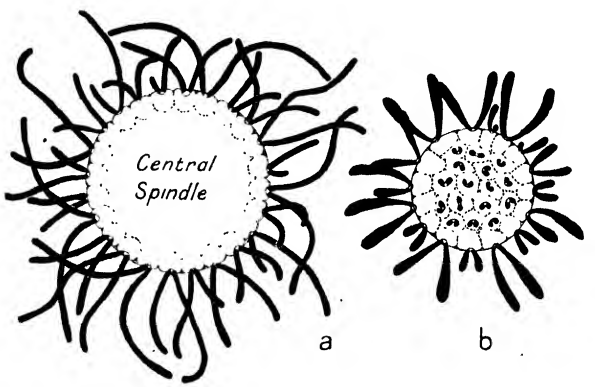

FIG. 4.—' Polar views ' of chromosomes and spindle at metaphase ; *a* in the Salamander, where there are 24 large chromosomes which arrange themselves on the periphery, with a large central spindle element in the middle ; *b* in an organism where there are 16 large chromosomes and 16 microchromosomes which arrange themselves in the centre of the spindle.

chromosomes of the set are about the same size. Where there are considerable size-differences it is almost always the smaller chromosomes which occupy the centre of the spindle (Fig. 4*b* and *Frontispiece*, Figs. 2 and 4). All these types of arrangement can be explained if the generalized repulsion between the surfaces of the chromosomes referred to earlier persists throughout metaphase, keeping the chromosomes with

their associated spindle elements at a certain distance from one another.

In the case of the peripheral chromosomes it is clear that they are attached by a single short region to the spindle, so that their long arms float freely in the cytoplasm outside the spindle, perhaps covered by a layer of spindle-substance. The region of attachment corresponds to one of the constrictions seen during prophase; this constriction, the spindle-attachment or *centromere*, is thus a permanent cell-organ which, although visually similar to the other or *secondary constrictions*, behaves entirely differently. Although the smaller central chromosomes are usually entirely embedded in the substance of the spindle, they can be seen to have centromeres of exactly the same nature as the peripheral ones.

Where a chromosome has been broken into two parts as a result of irradiation by X-rays that part which contains the centromere becomes associated with the developing spindle at prometaphase, while the part lacking a centromere floats freely in the cytoplasm and never becomes attached to the spindle.[117, 180] There is thus some evidence for regarding the centromere as the only part of the chromosome which plays a part in organizing the gelation of the spindle-elements from the original nuclear sap; perhaps 'spindle-element-organizer' would be a clumsy but descriptive name for it.

The position of the centromere is constant for each individual chromosome, but may vary from one chromosome to another in the set. Thus in *Drosophila melanogaster* chromosomes I and IV have subterminal centromeres, while chromosomes II and III have median ones. Where the centromere is median the chromosome will have the shape of a V with two limbs of equal length; where it is submedian the two limbs will be unequal (Fig. 5). It was formerly believed that the centromere was terminal in many cases and a distinction was drawn between

'V-shaped' and 'rod-shaped' chromosomes. It is now known that the centromere is never quite terminal; in other words there are always two limbs to the V, only one may be so short as to be practically below the limit of optical resolution.[148, 180, 181]

In many cases of chromosomes with median or submedian centromeres there appears to be a minute granule in the centre of the centromere which stains with aniline dyes and Haematoxylon; it resembles the minute granule which forms the short limb of 'rod-shaped' chromosomes. DARLINGTON [36] regards this as the actual organ of attachment and calls it the 'attachment chromomere'. I have, however,[180] given reasons for believing that it is the non-staining region which is the true attachment-organ; in many cases the granule cannot be seen in the middle of the non-staining region, although it may be below the limit of visibility in these cases.

Apparently the centromere, unlike the rest of the chromosome, remains undivided during prophase and only divides at prometaphase; its two halves then organize a spindle-element, above and below the equatorial plane. Up till now we have only been considering ordinary 'bipolar' spindles; but bipolarity is not an essential feature of the spindle—a fact which eliminates theories of mitosis based on a superficial analogy with electrical or magnetic models. In many cells such as those of cancerous tissues and in Sea-Urchin eggs which have been fertilized several times as a result of polyspermy, multipolar spindles with a number of equatorial planes intersecting one another are found [1, 10, 143]; there may be as many as 12 poles and six equatorial planes. The probable structure of these multipolar spindles is indicated in Fig. 3*b*.

Even more interesting than the multipolar spindles are the unipolar ones (half-spindles) found at meiosis in some insects (Fig. 3*c*) and described by the SCHRADERS [71, 162] and by METZ, MOSES and HOPPE.[125]

At present no useful suggestion can be put forward as to how they are formed.

So far we have said nothing of the ' spindle-fibres ' described in many text-books, but have considered the spindle as a bundle of ' elements ' corresponding in number with the chromosomes, with or without the addition of a central element between the centrosomes. There appears to be little doubt that many of the ' continuous ' or ' interzonal ' fibres described by various workers were in fact fissures between the separate elements. On the other hand, since the spindle almost undoubtedly consists of very long molecules which are all orientated parallel to its long axis it would not be surprising if it showed striations in the direction of the long fibre molecules. LEWIS and LEWIS [100] have shown that it is possible to produce ' spindle-fibres ' in living tissue-culture cells by the use of acid media ; the phenomenon is reversible, since on subsequently raising the *p*H the structures disappear.

We left the chromatids in prometaphase as a pair of thickened threads lying closely approximated throughout their length and only interrupted by the centromere and the secondary constrictions (if any). Usually they come to lie even closer together at metaphase, so that the visible ' split ' between them disappears ; a metaphase chromosome in transverse section thus has the shape of the symbol ∞. By most ordinary methods of fixation the metaphase chromatids show no trace of internal structure, but appear as homogeneous cylindrical rods (in some cases they are slightly club-shaped having a greater diameter at the distal ends than at the centromere. By various special methods of fixation, however (fixing in boiling water,[156] squeezing the chromosomes under a cover-glass,[31] exposing them to fumes of ammonia or strong acids [96, 98]) it is possible to show that each chromatid has a spiral structure, the apparent cylinder being a spring in which the successive gyres

are in contact (Fig. 2*d*). There is no doubt that this is the true structure in the living state ; all that the special methods of fixation have done is slightly to separate the gyres and thus reveal the spiral. The two chromatids are coiled independently (in other words their gyres do not interlock as happens when two parallel wires are wound round a cylinder) and in the same direction (right- or left-handed) at any one level ; the direction of coiling may change at the centromere, but does not necessarily do so. There has been a certain amount of controversy as to whether the direction of coiling is always the same for a particular chromosome or chromosome region. Apparently it is not.[183]

The existence of a spiral structure in metaphase chromosomes was discovered as early as 1880 by BARANETSKY and there seems no doubt that it is universally present both in plants and in animals.

The occurrence of a metaphase spiral explains the contraction and condensation process during prophase ; this must now be interpreted as due to the development of the metaphase spiral. It will be remembered that in nuclei whose resting stage is of short duration, the early prophase chromosomes are also spiralized. We have therefore two kinds of spirals, those of early prophase and those which develop at the end of prophase and are completed by metaphase. The metaphase spirals are, however, not a continuation of the early prophase ones, since the latter have disappeared by mid-prophase ; as a matter of fact the reverse is the case ; that is to say, the early prophase spirals are the remains of the metaphase spirals of the *previous division* which have persisted through the intervening resting stage, and only finally unwind in the mid-prophase of the next division if the resting stage is short (if it is protracted they may completely unwind before the beginning of prophase). DARLINGTON [34] calls the early prophase spirals *relic spirals*.

There are three ways in which the metaphase spiral might develop during late prophase and prometaphase. There is, unfortunately, no direct evidence as to which of these actually occurs, since observations on the origin of the metaphase spiral are very incomplete, the internal structure of the chromatids being difficult to study at this period.

According to the first method the chromosome rotates in order to become spiralized (either one end remains fixed and the other rotates or both ends rotate in opposite directions or the centromere remains fixed and the ends rotate). DARLINGTON [34] has given several reason why this cannot be the mode of origin of the metaphase spirals. According to the second method an *internal compensating spiral* (whose twists are below the limit of resolution of the microscope) develops in the opposite direction to the main one (i.e. right-handed if the main one is left-handed and vice-versa) and with the same number of turns as the main one. In order to understand this internal compensating spiral one can carry out a simple experiment with a piece of copper wire : fix the two ends in a pair of vices and wind the middle part into a spiral round a metal rod : the compensating spiral will easily be seen. DARLINGTON believes in the existence of this compensating spiral (which he calls the *molecular spiral*) and regards it as causing the development of the metaphase spiral. According to the third method (which is possible in a colloidal body like a chromosome, but not in a piece of copper wire) there is no internal compensating spiral ; a sliding of molecules on one another takes its place. It does not appear possible to decide at present which of the second and third alternatives is actually found in the chromosome.

4. FIRST STAGE OF ANAPHASE

Metaphase is a period during which almost no appreciable change takes place in the cell : it is

nearly always one of the shortest stages of mitosis. At the end of metaphase the halves of the centromeres (the latter having divided at prometaphase) appear to repel one another. At any rate the proximal ends of the chromatids (those, that is to say, which are attached to the spindle) begin to diverge and to move up the sides of the spindle towards the poles (Fig. 2*d*). From the mode of travelling of the centromere and in view of the fact that the spindle itself does not undergo any change of shape at this stage, the hypothesis of an active repulsion between the divided centromeres is the only possible one. Certainly there is no evidence for a ' traction of fibres ' at this stage—the movement of the chromatids is autonomous and depends on the centromeres. Why this repulsion-force should not manifest itself earlier is not clear ; perhaps the division of the centromeres is not finally completed until the end of metaphase.

As a result of this movement of the chromatids the attachment regions of the latter move up the spindle towards the poles until in most cases they have travelled about two-thirds of the distance from the equator to the poles. Where the chromosomes are short this means that the split halves are now completely separated ; where they are long the distal ends (those farthest away from the centromeres) will be still in contact (Fig. 2*e*).

5. Second Stage of Anaphase

When the autonomous movement of the chromatids has come to an end a remarkable change in shape takes place in the spindle (Fig. 2*e*). Its middle region between the two groups of centromeres undergoes elongation so as to complete the separation of the two sets of chromatids (which must now be called chromosomes). The growth and elongation of the middle region of the spindle to form a *stem body* is apparently a universal feature of mitosis [5] ; unfortunately we have no idea what it is due to. The stem-body is

clearly a solid gel like the rest of the spindle ; under abnormal conditions (e.g. in some hybrids and in cells cultured in hypertonic solutions) it may go on growing until the spindle is forced by lack of space to curl round in the cell (Fig. 3*d*). Usually the stem-body shows conspicuous longitudinal striations which are probably remnants of the divisions between the original spindle-elements.

In many cases a number of small granules make their appearance across the middle of the stem-body. They are known as the mid-bodies of the spindle, but very little is known of them. Usually they are not visible before late anaphase or telophase. In a few species of insects and arachnids large and conspicuous bodies make their appearance at anaphase half-way between the separating chromosomes. The best example of these ' equatorial bodies ' is in the mite *Pediculopsis graminum* [24] where they occur during oögenesis and in the early cleavage divisions. It is probable that these bodies represent masses of excess nucleic acid extruded from the chromosomes ; but since they give a negative reaction to the Feulgen test they cannot be composed of desoxyribose nucleic acid.[189] Similar bodies are formed during the female meiotic divisions of many moths and Trichoptera.

6. Telophase

The two groups of ' daughter chromosomes ' never actually reach the poles of the spindle, although as a result of the elongation of the stem body they may travel farther apart than the original distance between the poles of the metaphase spindle. When the cell has reached the stage represented by Fig. 2*e* the polar caps of the spindle disappear by a process of gel-solution ; the stem-body, on the other hand, frequently persists for a long time, even after cell division has been completed (Fig. 2*f*). As the polar caps of the spindle are destroyed a new nuclear membrane is formed round each of the telophas

groups of chromosomes. The details of the process whereby the cytoplasm becomes divided into two daughter cells are outside the scope of this book.

The changes which take place inside the nuclear membrane of the daughter nuclei during telophase are rather complicated but may briefly be described as a reversal of those which take place during the latter half of prophase—that is to say, de-condensation and de-spiralization. As a result of the latter the chromosomes become elongated and thrown into tight zig-zags inside the nuclear membrane. As they pass into the resting stage they become once more hydrated and lose their ' fixability '.

It will be remembered that we described the early prophase chromosomes as longitudinally split or divided into two chromatids. The telophase chromosomes, on the other hand, are probably unsplit and thus consist of a single chromatid each. The division of the chromosome in preparation for the next division usually takes place during the resting stage.*

The whole process of mitosis usually takes several hours from start to finish. From 2–24 hours is probably the usual range of variation in most organisms. In special cases, however (see Chap. I), it may take much longer. Prophase is nearly always the longest stage, prometaphase, metaphase and the two parts of anaphase are all short stages, while telophase is considerably longer, but usually not so long as prophase.

To sum up : mitosis consists of a series of cyclical colloidal phenomena, each of which is reversible. The main ones, so far as the chromosomes are concerned, are hydration, de-hydration, nucleination,

* Many workers disagree with this view, believing that the division takes place at an earlier stage, i.e. during the previous division. In all probability there is no uniformity in time of division as between different tissues and organisms. In some instances, at any rate, it is certain that anaphase and telophase chromosomes are already split in preparation for the next division.[97]

spiralization, de-nucleination and de-spiralization. Growth of the chromosome substance is probably an irreversible process and leads to the formation of two chromosome sets from whiat was originally a single set. The actual longitudinal division of the chromosomes probably takes place during the resting stage when they are invisible (or at any rate un-fixable) but the split halves (chromatids) remain closely approximated, due to the existence of a force of attraction between them, up to the beginning of anaphase. The formation of the spindle and the behaviour of the chromosomes at metaphase and anaphase depend on a special part of each chromosome, the centromere, which persists throughout the mitotic cycle and is a self-perpetuating cell-organ with pecular properties.

CHAPTER III

SPECIAL PROBLEMS OF MITOSIS

Number, Form and Size of Chromosomes

THE number of chromosomes in the somatic nuclei of an organism is usually the same for all the tissues and for all the individuals of the same species. There are exceptions to both these statements (i.e. organisms with different chromosome numbers in different tissues and species with different chromosome numbers in different 'varieties') but they need not be considered at present. The number of chromosomes in a somatic nucleus is usually even and is referred to as the *somatic number*. Where there are size-differences between the chromosomes of a somatic set it will usually be found possible to arrange them in pairs (Fig. 5), the two members of each pair being exactly alike in size, in position of centromere and (where they exist) of secondary constrictions. The complete set of chromosomes is thus made up of

two identical *haploid sets*. Organisms in which this is so are called *diploid organisms* and the somatic set may be called the *diploid set*. Sometimes even in diploid organisms one pair of chromosomes are unequal in size (Fig. 5) and sometimes the diploid number is uneven in one sex, there being a chromosome which does not form a member of a pair in that sex (Fig. 5, *f*, *g*). In these cases the uneven pair or the odd chromosome are sex-chromosomes. Their behaviour will be considered later. In hybrids between species whose chromosome sets differ it is naturally not possible to arrange them in pairs since the two haploid sets in the hybrid are not identical.

The two chromosomes of a pair are said to be *homologous*, since they contain the same series of genes arranged in the same order. The concept of homology is one which may be applied to parts of chromosomes as well as to whole ones, since one sometimes finds a pair of chromosomes which are homologous in some regions but not in others (see below).

In some organisms the chromosomes can be grouped, not into pairs, but into threes, fours or groupings of

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FIG. 5.—Somatic chromosome sets of various organisms. *a* after MAKINO [108], *b* after DARLINGTON [36], *d* and *e* after KIHARA and YAMAMOTO [85], *f* and *g* after HUGHES SCHRADER [70], *i* after MATSUURA and SUTO (*J. Fac. Sci.*, Hokkaido Imp. Univ. Ser., V, **5**, 33), *j* after MORGAN [127], the rest original. All figures slightly re-drawn. *b*, *d*, *e*, *i* are plants, the other organisms are animals. In *b* the coiling of the chromatids round one another is noticeable, in *i* the chromatids are parallel. The centromeres cannot be seen in *c*, *f*, and *g*, but are visible in all the others. In *a*, *c*, *f*, *g*, and *j* no trace of a 'split' between the chromatids can be seen, in the others it can be seen, either at the ends of the chromosomes, or throughout. *a* = Frog (*Rana temporaria*), *b* = *Pushkinia libanotica* (plant), *c* = Chicken, *d* and *e* = *Rumex acetosa* (Sorrel-Dock), *f* and *g* = *Icerya purchasi* (Scale-insect), *h* = *Chorthippus parallelus* (grasshopper), *i* = *Agapanthus umbellatus* (plant), *j* = *Drosophila melanogaster* ('closed-X' stock, with two ring-shaped chromosomes).

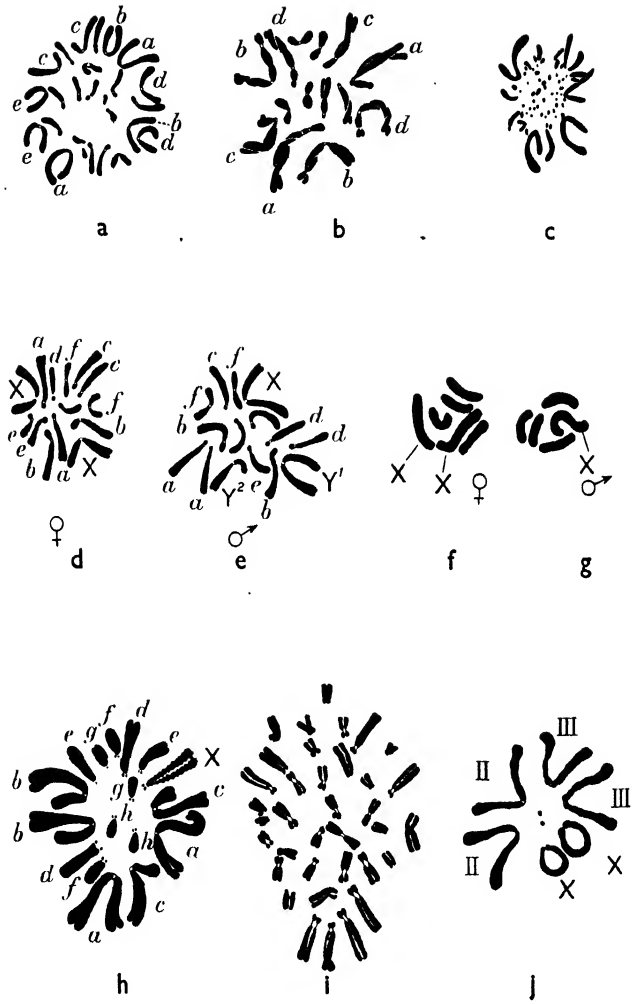


FIG. 5.

higher numbers. Such organisms (in which the somatic number is not diploid) are called *polyploids*, those with three of each kind of chromosome being *triploids*, those with four *tetraploids*, and so on (pentaploids, hexaploids, heptaploids, octoploids, &c.).

The lowest diploid number found in any organism is 2, which occurs in the Roundworm, *Ascaris megalocephala* var. *univalens* (this species also has another variety, *bivalens*, with 4 chromosomes in the diploid set).* The highest somatic numbers hitherto recorded are 224 for the moth *Phygalia pedaria* and 308 for the mulberry, *Morus nigra*.† Between 2 and 100 nearly all possible numbers are found in at least one species of animal or plant. Diploid numbers between 12 and 32 are common, those above and below these figures being progressively rarer. Both in animals and plants the commonest diploid number is 24.

As regards size the smallest known chromosomes are approximately 0·25 μ in length and about the same breadth at the metaphase of mitosis [177]; the longest ones are about 25 μ long and 2 μ in width.

Normally each chromosome possesses only a single centromere. By irradiation with X-rays it is, however, possible to cause two chromosomes to fuse in such a way that a compound chromosome is formed with two centromeres (Fig. 6 *a* and *b*). Such a chromosome may behave in either of two ways at mitosis [117]; either both centromeres in each chromatid may go to the same pole at anaphase or to opposite poles (if those in one chromatid go to opposite poles,

* These numbers for the two varieties of *Ascaris megalocephala* refer only to the germinal tissues. In the somatic tissues a much larger number are found, as a result of fragmentation of the 2 or 4 originally present in the fertilized egg (see later).

† Some Protozoa (e.g. *Aggregata*) appear to have even more than this.

naturally those in the other will do likewise). When the centromeres in a chromatid go to opposite poles the part of the chromatid between them will be stretched out and eventually broken. Apparently each of the two ways of division happens in 50 per cent of cases; the number of chromosomes with two centromeres is thus progressively reduced in the course of a few divisions, and such chromosomes stand no chance of becoming permanent.

One case exists, however, in which normal chromosomes have more than one centromere each. That is in *Ascaris megalocephala* where the middle region of the long chromosomes found in the 'germ-line' appears to contain about sixteen separate centromeres (Fig. 6c). Owing to the fact that these are very close together all those in one chromatid are forced to go to the same pole at anaphase.[182] This happens in the spermatogonial and oögonial divisions and at the first cleavage division. In the later cleavage divisions however, a different type of division takes place in all those blastomeres which are going to give rise to the somatic tissues of the adult—here the central region of the chromosomes breaks up into a number of much smaller chromosomes, each of which probably has a single centromere. The ends of the chromosomes are left with no centromeres and do not become connected with the spindle in any way but degenerate in the cytoplasm. This process results in an organism whose germ-cells contain two or four (according to the variety) large chromosomes, while the somatic cells have a much larger number of small chromosomes (the exact number of which is still in doubt). The germ-line chromosomes of *A. megalocephala* may be referred to as *polycentric* in contrast to the ordinary type of *monocentric* chromosome.

In another species of *Ascaris*, *A. lumbricoides* the ends of the chromosomes are broken off and degenerate in the cytoplasm in the same way, but there is no fragmentation of the central region, so that the

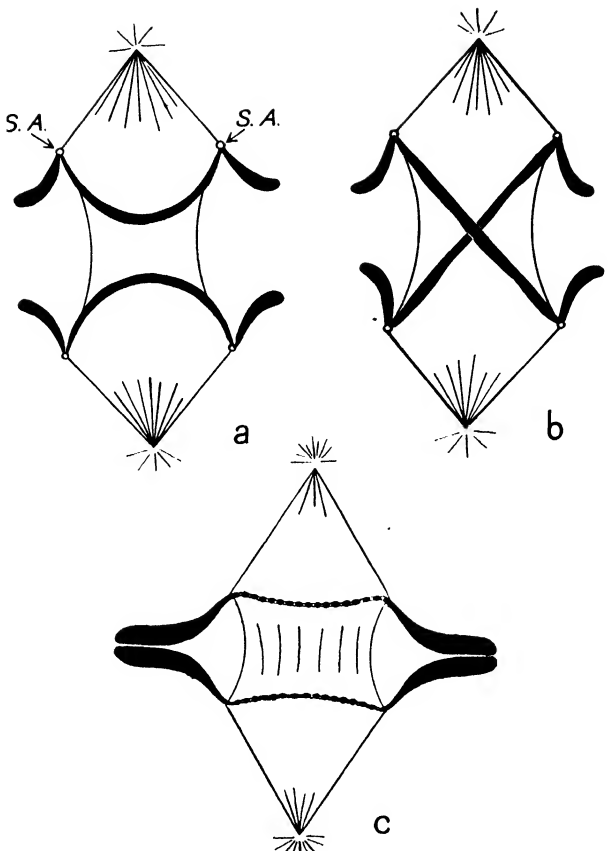


FIG. 6.—*a* and *b*: diagrams to show the two alternative modes of behaviour of a chromosome with two centromeres at anaphase; *c*: a diagram of the method of anaphase-separation in one of the germ-line chromosomes in *Ascaris megalocephala*; the numerous centromeres are situated so close together that those in one chromatid always go to the same pole.

chromosome number of the somatic and germinal tissues is the same.[12]

Two other types of chromosomes may be mentioned here which have been observed on a number of occasions, but do not appear to have become permanent in any wild species of organism. The first of these is the *branched chromosome*. It was formerly believed on genetical evidence that the '*pale*' mutation of *Drosophila melanogaster* was due to a small piece of the second chromosome having been broken off and attached to the side of the third one. This explanation has now been abandoned as far as the pale mutation is concerned, but undoubted cases of branched chromosomes have been seen in several organisms.[27, 99] In most of these cases, the side-branch was joined on to the main chromosome at the centromere.

The other type of chromosome is the *ring chromosome*, which has been found on a number of occasions, both in normal cells (in which ring chromosomes have apparently arisen spontaneously) and in X-rayed material.[135, 103, 180] Here the two ends of the chromosome are fused together so that a closed ring results. In *Drosophila melanogaster* there exist stocks in which the X-chromosome has its proximal and distal ends fused so as to form a ring (Fig. 5*j*).[127] In these cases the two rings which separate from one another at anaphase may become interlocked like two links of a chain. This may lead to both chromatids going to the same pole instead of to opposite poles.

During prophase and metaphase the chromatids of which the chromosome is composed are held together in a *paired* condition throughout their length (Fig. 2). It appears (and observations on meiosis confirm this) that they are held together, not merely in a mechanical way (such as would result from a common investing sheath—like two sausages in a skin) but by a force of mutual attraction between the homologous genes of which the chromatids are com-

posed. Speculation as to the nature of this force is outside the scope of this book; but at the moment it appears to be unparalleled in biological systems. Now, is this force exhausted when two chromatids are in contact, in the paired condition, or does it extend to other homologous chromatids (in other words is the force exerted merely between pairs of genes, or between threes and fours, &c.) ? In diploid nuclei at prophase and metaphase each chromatid is represented four times and if there was some 'residual' attraction we should expect homologous chromosomes to lie side by side in close approximation. Usually this is not the case, i.e. there is little or no residual attraction, but it is exactly what does happen in the two-winged flies (Diptera) including *Drosophila* where the homologous chromosomes lie side by side (but not actually touching) during prophase and metaphase. This state of affairs is called *somatic pairing*; there is apparently sufficient residual attraction to cause it to develop even in triploid nuclei. Apart from this and a few other similar cases chromosomes always keep at a certain distance from one another throughout the mitotic cycle as a result of the surface repulsion force already referred to.

Genetically Active and Genetically Inert Chromosomes

No attempt will be made here to explain how the idea that the genes are arranged in linear order along the chromatids has been proved, since the matter is dealt with fully in all text-books of genetics. Certain special problems must, however, be gone into. It has long been known that the Y-chromosome in *Drosophila melanogaster* is genetically almost inactive. Males lacking it (called X-nought males) are viable but sterile. It has been shown that the Y contains a number of regions necessary to ensure fertility in addition to the normal allelomorph of the gene

'bobbed' [168]; apart from these it may contain a few other genes. In Maize there is also a chromosome (the B-chromosome) which contains no known genes and may be present any number of times in the chromosome set or may be absent altogether without in any way affecting the phenotypic appearance of the plant.[104] These, then, are examples of *inert chromosomes*. It has been shown [131] that the part of the X-chromosome in *D. melanogaster* which is next to the centromere (about one-third of the total length) is almost completely inert (up to the present this region only contains one known gene, namely bobbed, which suggests that this region is homologous with that part of the Y which likewise contains bobbed). In addition there are a number of other short regions in the autosomes which appear to be inert: the largest of these are situated on either side of the centromeres in chromosomes II and III. The possibility thus arises that in many other organisms some chromosomes or regions of chromosomes may be genetically inert; for this reason we must not expect the number of linkage groups and the haploid number of chromosomes to correspond in all cases.

Salivary Gland Chromosomes in Diptera

In the nuclei of the salivary glands in the Diptera (and also in the nuclei of some other tissues such as the gut-epithelium and the Malpighian tubules, which show an essentially identical structure) the chromosomes appear as enormously enlarged threads whose structure could not for a long time be interpreted in terms of ordinary mitotic chromosomes. The first clue to the analysis came when it was realized that in the salivary gland nuclei the phenomenon of somatic pairing is developed to the point where the homologous chromosomes are completely in contact throughout their length.[64] As a matter of fact it is often possible by means of crushing under a cover-glass to separate slightly the homologous chromosomes; when this is

done it will be seen that they are spirally wound round one another as in a piece of two-strand rope.[92] The number of these threads thus corresponds to the haploid, and not to the diploid number of chromosomes, but in *Drosophila* the two arms of V-shaped chromosomes are represented by separate threads; thus in the female *D. melanogaster*, with eight chromosomes in the diploid set, four of which are V-shaped, the salivary gland nuclei contain six threads (i.e. one representing the two X-chromosomes, one for each of the arms of the IInd and IIIrd chromosomes and a short one representing the two IVth chromosomes). In *Drosophila* all these six threads are attached at one end to a body called the *chromocentre*, to which the nucleolus (arising from the 'nucleolar organizers' in the proximal regions of the X and Y) is also attached. In many other Diptera (e.g. *Bibio* and *Chironomus*) there is no chromocentre, each chromosome, whether rod-shaped or V-shaped, being represented by a separate thread.[2, 64] Apparently the chromocentre results in part from a fusing together of the centromeres and adjacent regions of all the chromosomes.

The nuclei of the salivary gland cells are very large (about 25 μ in diameter in *Drosophila*) but even so the chromosomes are so long that they are tangled and coiled up so as to pack them inside the nuclear membrane. The usual method of studying these chromosomes consists in fixing and staining them simultaneously with a saturated solution of carmine in 40 per cent acetic acid. The preparations are then lightly crushed under a cover-glass in order to rupture the nuclear membranes and spread out the chromosomes on the slide. This crushing results in a considerable stretching of the chromosomes; the X-chromosome of *D. melanogaster* may be 260 μ long in acetocarmine preparations, while in the living salivary gland nucleus it is probably only about 150 μ in length. Even so the unstretched chromosomes in the salivary gland nuclei are at least 50 times

as long as the chromosomes at ordinary somatic mitoses and 1,000 times their volume.

Each of the homologous chromosomes which pair to form the threads in the salivary gland nucleus is transversely striated with bands which stain with ordinary nuclear dyes (e.g. Crystal Violet and Haematoxylin). These cross bands are of varying thickness and are separated by non-staining internodes. It is apparently these internodes which stretch when the nucleus is crushed. The bands correspond exactly in position in the two homologous chromosomes and are always the same for a particular chromosome in different individuals of the same species. The thicker bands as seen in the uncrushed nuclei are apparently made up of several thinner bands which can be separated by crushing and stretching under a cover-glass. The total number of bands in the X-chromosome of *D. melanogaster* is at least 1,000 if one counts all the finer bands of which the thicker ones appear to be made up.[92] Each band is clearly a disk, that is to say they extend through the thickness of the chromosome ; moreoever each disk or band is made up of a number of granules which have more or less completely fused to form a transverse plate ; in *Chironomus* there are at least 256 of these granules in each band. The granules in one band are connected with those in the next by means of fine longitudinal threads which run through the non-staining internodes. The two strands of our ' rope ' are thus themselves made up of a number of threads which bear periodic enlargements in the form of granules that tend to fuse into transverse bands.[92]

The salivary gland chromosomes are thus to be regarded as resting stage or early prophase chromatids stretched out straight which are not wound into a tight spiral as at an ordinary somatic metaphase and which have split longitudinally again and again. All the peculiarities of the salivary gland nuclei can be explained on the basis of these two simple assumptions

if one takes into account the well-known somatic pairing phenomenon found in all Diptera. The fact that the salivary gland chromosomes are about fifty times the length of the ordinary metaphase chromosomes will be understood if one takes a tightly coiled spring and pulls it out straight. The great volume of the salivary gland nuclei (about 65,000 μ^3) is natural if they are polyploid and the thickness of the chromosomes results from the fact that they have divided a large number of times.

The formation of the chromocentre has been shown [149] to be due to the fact that the regions immediately adjacent to the centromeres (which are inert but all ' homologous ') have, as a result of their homology, all fused into a single mass (Fig. 7*d*). The origin of the chromocentre is thus ultimately the result of the somatic pairing phenomenon. *Drosophila* species with extensive inert regions have large chromocentres, while those with shorter inert regions have small chromocentres.[49] On the other hand many Diptera which lack a chromocentre altogether possess extensive inert regions in their chromosomes. In *Drosophila* species the chromocentre contains a number of bands derived from the inert regions—these bands are much fainter than those of the ' active ' regions and have a peculiar appearance. Portions of the inert regions which have been transferred (by inversion or translocation) to a new situation in the chromosome usually participate in the formation of the chromocentre, unless they are very short.

Sex Chromosomes

All chromosomes other than sex chromosomes are called *autosomes*. The sex-determining mechanism of the majority of bisexual animals and plants consists of a pair of chromosomes which may be regarded as modified autosomes. In one sex these form an equal pair of homologous chromosomes while in the other the pair is unequal. The sex which possesses

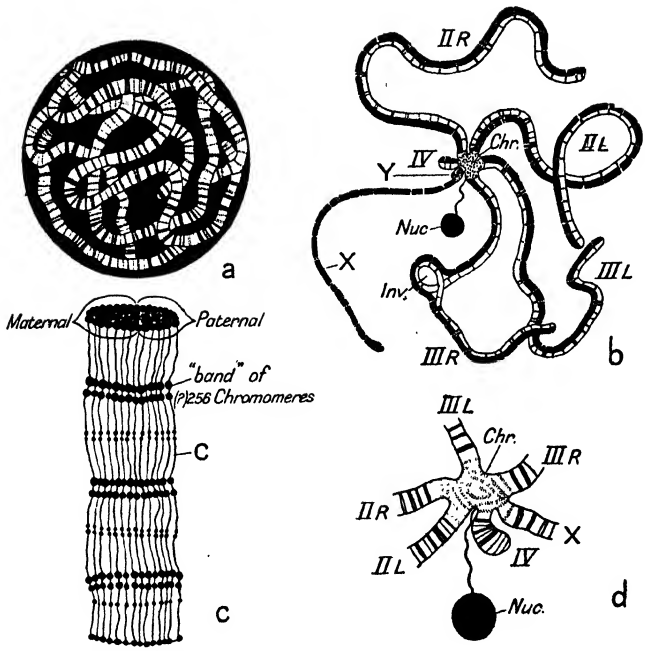


FIG. 7.—Diagrams illustrating the structure of salivary gland chromosomes. *a* = a general view of a salivary gland nucleus with the chromosomes coiled within it. The ' bands ' are shown, but not the threads connecting them. The nuclear sap is shown in black. *b* = the chromosomes of a salivary gland nucleus in the male *Drosophila melanogaster* spread out by crushing the nucleus. The maternal parts of the paired chromosomes are shown in black, the paternal parts white. Chr. = chromocentre (stippled); Nuc. = the nucleolus. II L and II R are the two arms of the IInd chromosome, III L and III R the two arms of the IIIrd chromosome; the IVth chromosome and the X and Y chromosomes (the latter very small) are also shown. An inversion (Inv.) is shown in the 'right' arm of the IIIrd chromosome. *c* = a diagram of a small part of a salivary gland chromosome, composed of 256 threads, 128 paternal and 128 maternal. *d* = a diagram illustrating how the chromocentre is made up (in the female of *D. melanogaster*) by a fusion of the inert parts of all the chromosomes (represented by stippled bands).

the unequal pair is called the *heterogametic sex* since it produces two kinds of gametes or spores ; the other sex is called the *homogametic sex*. In most groups of organisms it is the male which is the heterogametic sex (that is to say there are two kinds of sperms or pollen grains and only one kind of egg or megaspore), but in some groups it is the female which is heterogametic—that is to say there are two kinds of eggs or megaspores, all the sperms or pollen grains being alike (see Table I). In the Bryophyta (mosses and liverworts) where the sexual stage of the life cycle is haploid the male and female gametophytes each contain one member of a pair of sex chromosomes.

It will be seen that the sex-determining mechanism is merely a special kind of heterozygosity—in respect of a whole chromosome instead of in respect of a single gene. The sex chromosomes are in most cases (perhaps in all) not the only ones bearing sex-determining genes ; probably all the autosomes carry genes which are concerned with the development of characters of one or the other sex ; all that the sex chromosomes do is to act as a differential mechanism which switches the development of the embryo over to maleness or femaleness from a potentially hermaphrodite condition.

According to the usual terminology the chromosomes forming the equal pair in the homogametic sex are called X-chromosomes. The diploid set in the heterogametic sex contains one X-chromosome and in addition a chromosome bearing a greater or less resemblance to it called the Y-chromosome. Some authors use the letters Z and W instead of X and Y where it is the female sex which is heterogametic. We can regard the various types of sex chromosomes as progressive evolutionary modifications of an original pair of autosomes, these modifications consisting of the XY pair becoming more and more unlike. This is a simplification of the true state of affairs, however, since the evolutionary trans-

formations which the sex-chromosome mechanism has undergone are very complex in some groups (see Chapter VI). In the majority of fishes and amphibia there is no visible cytological difference between the X and the Y ; they probably only differ in respect of a few genes. Thus in fishes of the genus Platypoecilus one species has male heterogamety, another female heterogamety.[8] In most mammals and in many insects (such as *Drosophila melanogaster*) the X and the Y are of very different sizes so that they can easily be distinguished. Here it is probable that only a short region of the Y is homologous to a similar short region in the X. Usually the Y is smaller than

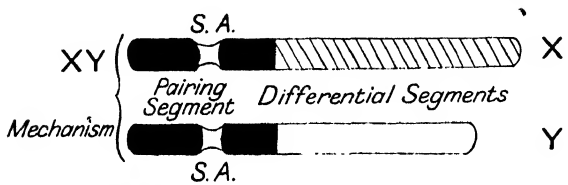


FIG. 8.—Diagrams of the sex-chromosomes of the male rat. S.A. = Centromere.

the X but in some cases, as in *D. melanogaster* it is considerably longer. In many organisms the Y is very small indeed and in a large number of groups it has been lost altogether. In these the diploid set in the heterogametic sex consists of an odd number of chromosomes (one less than in the homogametic sex).

In a number of organisms the X is represented by two separate chromosomes which can be called X_1 and X_2. Thus in some Praying Mantids, e.g. *Mantis religiosa*, *Tenodera sinensis*, *Stagmomantis carolina* and *Sphodromantis viridis* (see *Frontispiece*, Fig. 1), the chromosome sets of the two sexes are :

Male X_1, X_2, Y
Female X_1, X_1, X_2, X_2

In this case it will be seen that the heterogametic sex

has an odd number of chromosomes, but a Y is present.[88, 184, 187] The two ends of the Y are probably homologous to regions in the X_1 and X_2 respectively.

In the stone-fly, *Perla marginata*,[80] and in the Thysanuran, *Lepisma*, there is a similar situation but a Y is absent so that the two sets are:

Male X_1, X_2
Female X_1, X_1, X_2, X_2

In some cases it is the Y which is represented by two separate chromosomes, there being only one X. Thus in the dioecious Sorrel Dock, *Rumex acetosa*, we have (Fig. 5*d*, *e*):

Male plant X, Y_1, Y_2
Female plant X, X.

In this case it will be seen that the heterogametic sex possesses one more chromosome than the other instead of one less as in the XX : X type.[85]

There are a few organisms in which the X is represented by even more than two chromosomes. In the Assassin-Bugs (family Reduviidae) there may be as many as five different X's. Thus in *Acholla multispinosa* the situation is:

Male animal 10 pairs of autosomes,
$X_1X_2X_3X_4X_5Y$
Female animal 10 pairs of autosomes,
$X_1X_1X_2X_2X_3X_3X_4X_4X_5X_5$

In the Bed Bug (*Cimex lectularius*) there is only one Y-chromosome, but the number of X's varies from three to fifteen in the male.[39] Most of these X-chromosomes are probably inert 'supernumeraries' which play no part (or only a very subsidiary part) in sex determination.

The identification of sex-chromosomes is naturally difficult when the X and Y are cytologically indistinguishable. Where they are visibly different it is usually possible to identify them by careful compari-

son between the somatic chromosome sets of the two sexes. In the more highly evolved types of sex-determining mechanism the sex-chromosomes, or at any rate parts of them, are often clearly recognizable by the fact that they become nucleinated at a different rate from the autosomes. Thus in the spermatogonial mitoses of the Acrididae and Gryllidae (Grasshoppers

TABLE I

Male Heterogametic and Female Homogametic.	Female Heterogametic and Male Homogametic.
Animals	
All insects except	those with haploid males and the Lepidoptera and Trichoptera [165, 90]
Crustacea, Arachnida, Opisthogoneata, Annelida, Nematoda, Echinodermata (as far as known) [161]	
All Vertebrates except	Some Fishes [8] Some Reptiles at any rate [140] All Birds [177, 167]
Plants [161]	
Rumex section Acetosa, Humulus, Melandrium, Populus, Empetrum, Elodea	Hexaploid *Fragaria elatior* [101]

TABLE II

Organisms with Sex-Determination by Male Haploidy

Insects: *Rhynchota*
- Some Aleurodidae [171]
- Some Coccidae [162, 68, 69]

Hymenoptera
- Probably all [157, 172, 134]
- *Coleoptera: Micromalthus debilis* [164]

Arachnida: *Acarina* (Mites)
- Some at least [160, 145, 24]

Rotifera: *Aslanchna* [190]

(Also possibly in some *Thysanoptera*)

and Crickets) the greater part of the X has only reached the mid-prophase degree of nucleination by the time the autosomes are in metaphase.[141, 180] This process of differential condensation is known as *heteropycnosis*. In the above case we can speak of *negative heteropycnosis*; the opposite condition of *positive heteropycnosis* (where the sex-chromosome reaches the metaphase degree of nucleination when the autosomes are still in early prophase) is often found in the first meiotic division (see Chap. V).[7, 183] In the short-horned Grasshoppers and Crickets the X-chromosome undergoes a complete reversal of heteropycnosis in the course of spermatogenesis, but in some other groups (such as the Long-horned Grasshoppers) it is always positively heteropycnotic and does not undergo a reversal of behaviour.[183, 189]

Heteropycnosis is not confined to sex-chromosomes; thus in many species of grasshoppers there is one autosome which shows strong positive heteropycnosis in about two-thirds of its length at the first meiotic division.[18] Moreover in *Drosophila melanogaster* all the inert regions show heteropycnosis at the somatic divisions. There seems to be some sort of connexion between inertness and heteropycnosis, and it is probable that all heteropycnotic regions are more or less inert and vice-versa.

In a number of groups of organisms sex-determination does not depend on a pair of sex-chromosomes, but on whether the eggs are fertilized or develop parthenogenetically. Thus in these cases the males are haploid, the females diploid. This is the case in the Hymenoptera, the Mites (some species at any rate) and in some Scale Insects (Coccidae). In the Hymenoptera it is clear from recent work on the parasitic wasp *Habrobracon* that the haploid-diploid scheme of sex-determination co-exists with a mechanism involving female heterogamety.[172] In order to be female an individual must be heterozygous for a particular gene or complex of genes. In-

breeding leads to the production of a certain number of diploid homozygotes which are males. Although the details of the genetical mechanism are not fully worked out yet it seems probable that haploid individuals are male because they are not heterozygous rather than because they are haploid. It is quite unknown whether male haploidy in other groups depends on a similar principle.

Polyploidy

Polyploid cells arise in the first place through a failure of cell division; that is to say that in a particular cell the chromosomes divide, but the cell fails to do so.[178, 179] If this happens in a diploid organism a tetraploid cell will result. Such a process occurs normally in a small percentage of the meristem cells in the root tips of certain plants [121, 126]; as a result patches of tetraploid tissue are produced, surrounded by diploid cells. In the tomato it is possible to produce tetraploid shoots by repeated cutting back.[79] From such shoots it is possible to produce tetraploid tomato plants.

If the process is repeated in a tetraploid plant octoploid cells will be produced. Triploids arise in the main through crossing between diploids and tetraploids, hexaploids by a doubling of the chromosome set in a triploid and pentaploids by crossing between tetraploids and hexaploids.

It has been found necessary to distinguish between two kinds of polyploidy which are called *auto-polyploidy* and *allo-polyploidy*. An auto-polyploid is an organism with more than two haploid sets of chromosomes which are all alike—it has arisen by doubling of the chromosomes in an individual which is not a hybrid. In an allo-polyploid, on the other hand the doubling has taken place in a hybrid, so that the several haploid sets are not all identical, having been derived from different parent species.

Polyploidy may occur within a single species, as in

the case of the Cruciferous plant *Biscutella laevigata* where diploid, triploid, tetraploid, pentaploid and hexaploid plants have been found [112] or as between different species of the same genus. A good example of the latter phenomenon is the Section Lapathum of the genus *Rumex* (Table IV). Intraspecific polyploidy is of course always auto-polyploidy, while the interspecific kind may be either auto- or allo-polyploidy. Recent work has shown that many somatic tissues of insects are highly polyploid, the chromosomes having divided repeatedly without any breakdown of the nuclear membrane or formation of a spindle (*endomitosis*). In the Pond-skaters of the genus *Gerris* it has been calculated that the largest cells in the salivary glands are 2,048-ploid, containing 22,528 chromosomes.

A special type of polyploidy occurs where one or more chromosomes, but not the whole haploid set, are present more than twice in the complete chromosome set; this is called *polysomy*. The most common type of polysomy is *trisomy* where one or more chromosomes are present three times, the others being only present twice. The opposite phenomenon where a polyploid lacks one or more chromosomes from one haploid set is called *aneuploidy*: it is obvious that there is no hard and fast distinction between polysomy and aneuploidy (see Table III).

TABLE III

If n = the haploid number of one species and *n* that of another, then—

2n = diploid number of first species
2n = „ „ „ second species
n + *n* = diploid number of hybrid between them
3n and *3n* = the autotriploids
4n and *4n* = the autotetraploids
2n + *2n* = the allotetraploid
2n + 1 = a trisomic
4n − 1 = an aneuploid
} these terms are partially interchangeable
4n + *2n* and 3n + *3n* = different kinds of allohexaploids

TABLE IV

SOMATIC CHROMOSOME NUMBERS IN THE GENUS RUMEX (SECTION LAPATHUM) [84, 77, 78]

R. salicifolius	20
R. alpinus	20
R. obtusifolius (one var.)	20
R. conglomeratus	20
R. sanguineus	20
R. scutatus	20
R. pulcher (one var.)	20
R. maritimus	40
R. limosus	40
R. brittanicus (one var.)	40
R. pulcher (another var.)	40
R. domesticus	40
R. obtusifolius (another var.)	40
R. crispus	60
R. patientia	60
R. orientalis	60
R. domesticus (another var.)	60
„ („ „)	80
R. japonicus	100
R. hymenosepalus	100
R. andraeanus	120
R. britannicus (another var.)	160
R. aquaticus	200
R. hydrolapathum	200

Owing to the fact that in bisexual organisms the sex-determining mechanism depends in general on the segregation of a single pair of chromosomes at meiosis polyploid species and varieties are usually found only in groups where reproduction does not depend on bisexuality (most Angiosperms, parthenogenetic and hermaphrodite animals such as the Oligochaeta, &c.). If polyploidy occurred in bisexual species it would completely upset the sex-determining mechanism.[130] Since the vast majority of Angiosperms are monoecious while the bulk of the Metazoa are dioecious polyploidy is far commoner in the higher plants than in animals.

CHAPTER IV

THE GENERAL OUTLINE OF MEIOSIS

MEIOSIS is the antithesis of fertilization; in diploid organisms it results in the chromosomes being reduced to the haploid number. If meiosis takes place at the beginning of the life-cycle, just after fertilization (this type of meiosis is called *initial* or *zygotic meiosis* and occurs in most of the Sporozoa and in the Charices,[50] Basidiomycetes [15] and Ascomycetes [195] among plants as well as in some of the lower Algae [63]) the result of fertilization followed by meiosis will be a haploid adult organism. If on the other hand meiosis occurs just before fertilization (i.e. during gametic formation) as happens in all the higher animals (Metazoa) then the adult organism will be diploid. In the higher plants with an alternation between the sporophyte and gametophyte generation meiosis takes place during spore-formation, i.e. occupies an intermediate place in the life-cycle; where the gametophyte generation is the predominant one (as in the mosses and liverworts) the 'adult' phase of the life-cycle will be haploid; where it is the sporophyte which is predominant (as in the higher plants) the 'adult' phase will be diploid. Thus a moss-plant is haploid and a buttercup diploid, but in both meiosis takes place at the same stage in the life-cycle, during spore-formation.

FIG. 9.—Diagrams of the main stages of meiosis. Two pairs of chromosomes AA′ and BB′ are shown, the A and A′ chromosomes being submedian centromeres, the B and B′ chromosomes having sub-terminal ones. Three chiasmata are formed in the AA′ bivalent, one in the BB′ bivalent. S.A. = centromere, Ch. = chiasma. There is no 'terminalization' of chiasmata. In the BB′ bivalent a rotation of the arms takes place, in the AA′ bivalent there is no rotation.

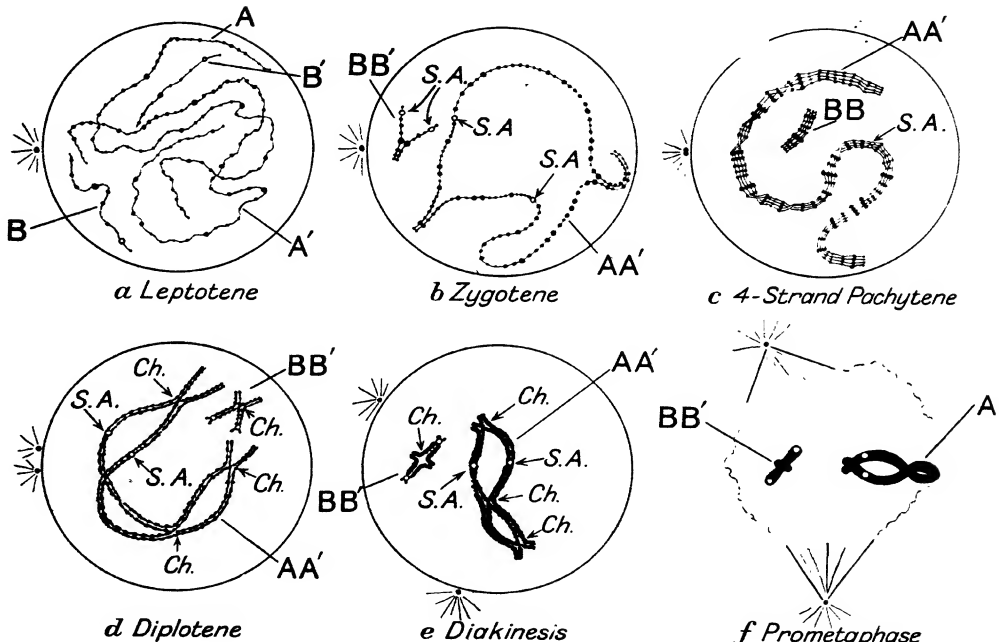

A
B′
B
A′
a Leptotene
BB′
S.A.
S.A
S.A
AA′
b Zygotene
AA′
BB
S.A.
c 4-Strand Pachytene
Ch.
BB′
S.A.
Ch.
S.A.
Ch.
Ch.
AA′
d Diplotene
Ch.
AA′
Ch.
S.A.
BB
S.A.
Ch.
Ch.
e Diakinesis
BB′
A
f Prometaphase

FIG. 9.

Meiosis has been defined by DARLINGTON [31] as 'the occurrence of two divisions of a nucleus accompanied by one division of its chromosomes'. The whole process must be regarded as having arisen in the first place through a profound modification of two mitotic divisions. It is very remarkable that in all essential details meiosis is the same wherever it occurs; it is consequently possible to give a general account of it which will apply equally well to the gametogenesis of an insect and the sporogenesis of a plant.

The first meiotic division always has an elongated prophase; since this is in many ways different from a mitotic prophase it is necessary to sub-divide it into a number of stages which, although they correspond to the early, mid- and late prophase stages of mitosis, have different names to indicate the main processes which take place. The names of these stages are, in order, *leptotene*, *zygotene*, *pachytene*, *diplotene* and *diakinesis*. After diakinesis (which corresponds to the end of prophase) comes a short prometaphase, followed by the metaphase of the first division ('First Metaphase').

In the following account we shall describe meiosis in a diploid organism; the meiosis of a polyploid is in some respects more difficult to understand and is best left until the details of the process in a diploid have been explained. As no mention will be made of the cytoplasmic phenomena of meiosis the description will do for either spermatogenesis or oögenesis, macro- or micro-sporogenesis, since there are no constant differences between the nuclear phenomena in the two sexes.

LEPTOTENE

This is the earliest part of the prophase of the first meiotic division—it corresponds to the very beginning of prophase in an ordinary mitosis. The chromosomes in the leptotene stage resemble those of the early

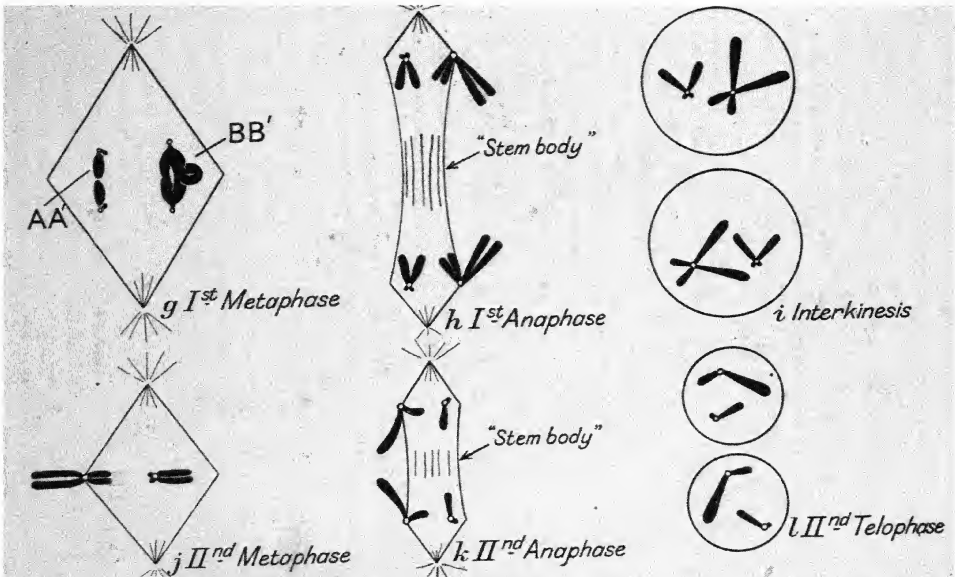


FIG. 10.—Diagrams of the main stages of meiosis (continued from Fig. 9). Metaphase of the First division to telophase of the Second.

prophase of mitosis except for one important feature ; *they are not longitudinally divided*—in other words each consists of a single chromatid and not of two chromatids held together throughout their length as in the case of mitosis. Some authorities claim to have observed a split in leptotene chromosomes, but its existence (or at any rate its visibility) seems very doubtful. Another point of difference which is somewhat variable is that the leptotene chromosomes are rather clearly made up of a series of granules (called *chromomeres*) connected by non-staining intervals ; this may also be the case at mitosis, but it is not usually so obvious. As in the case of the granules in the salivary gland chromosomes it has naturally been suggested that the chromomeres are actual genes. In various *Liliaceae* the total number of chromomeres in the whole chromosome set has been counted and found to lie between 1,500 and 2,500 [9] ; of course we do not know the total number of genes in these plants, but in *Drosophila* the total number (including the hitherto undiscovered ones) has been estimated at under 10,000 by various authorities.

The leptotene chromosomes are present in the same number as in the somatic tissues ; very often they are not arranged at random inside the nucleus but preserve the arrangement of the previous telephase (with all the centromeres together at one side of the nucleus and the chromosomes arranged as in a bunch of flowers [76]) ; in this case they are said to be polarized. In other cases they appear to have contracted into a tangled mass which lies to one side of the nuclear cavity.

Zygotene

Leptotene is usually a stage of short duration. It is followed by a stage called zygotene in which the homologous chromosomes come together in pairs and become closely approximated throughout their length. This process is called *pairing* or (in the older accounts)

synapsis. In the case of each pair of homologous chromosomes the pairing process begins at one or more points and then spreads along the length of the chromosomes (Fig. 9*b*). Where the telophase arrangement of the previous division has been retained pairing begins at the centromeres—otherwise it may begin anywhere. It must be pointed out that the pairing is not merely between homologous chromosomes, but always between strictly homologous regions; this can be seen very clearly where the chromomeres are distinct, since they are of slightly different sizes (Fig. 9*b*). If we call those in one homologous chromosome a, b, c, d, . . . and those in the other a′, b′, c′, d′, . . . then a will pair with a′ and b with b′ and so on. If a short region has become inverted in one homologous chromosome but not in the other (as sometimes happens) then the inverted region will remain as an unpaired loop in the middle (Fig. 11*a*). If a rather longer section is inverted the loop will twist round and pair as in Fig. 11*b*. If a small section is completely missing from one chromosome, then the corresponding section in the homologous chromosome will form a short loop (Fig. 11*c*).[31] It appears, therefore, that the force of attraction is a mutual one between homologous chromomeres (or genes) and that it is probably identical with the force which keeps the two chromatids of a chromosome together throughout their length in the prophase of mitosis. It seems natural that this force should lead to a pairing of homologous chromosomes at zygotene, since these have not undergone the usual longitudinal division—they are still unsplit at a stage when they would normally be split; the force of attraction thus satisfies itself at mitosis by maintaining chromatids together and at zygotene by bringing distinct chromosomes into longitudinal approximation. DARLINGTON regards the prophase of the first meiotic division as ' precocious ' in comparison with the prophase of an

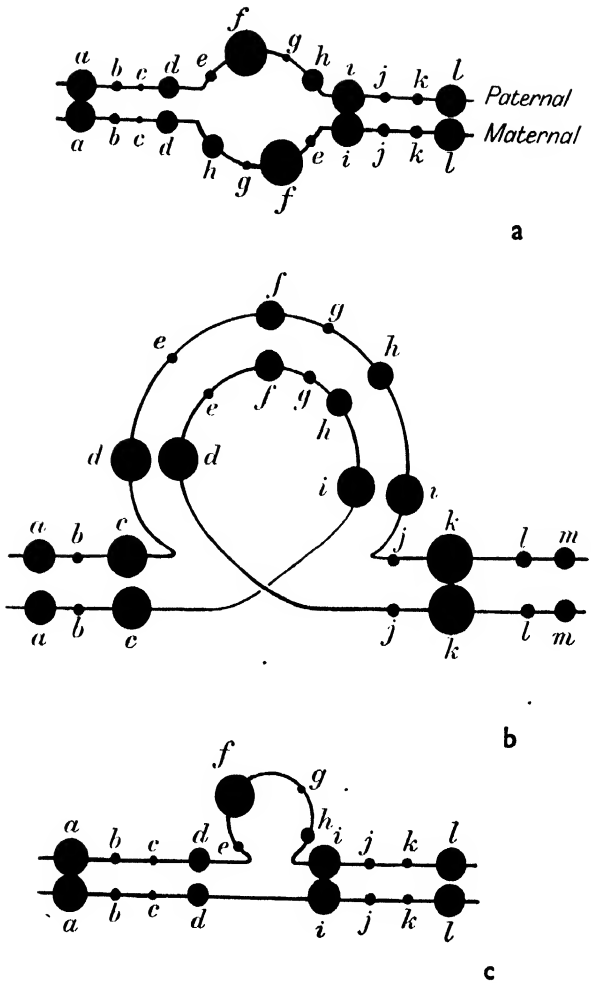


FIG. 11.—Diagrams of chromomeres (or genes) at the two-strand pachytene stage in a bivalent which is heterozygous for (*a*) a small inversion, (*b*) a large inversion, (*c*) a deletion.

ordinary mitosis, but it is probably better to regard the splitting of the chromosomes as being delayed.

Pachytene

As a result of pairing the apparent number of chromosome threads (in a diploid organism) has been reduced to half; if there were $2n$ chromosomes in leptotene there will be n associations of two chromosomes at the beginning of pachytene. These associations of pairs of chromosomes are called *bivalents*. Each bivalent has a split down the middle and thus closely simulates an ordinary mitotic chromosome at

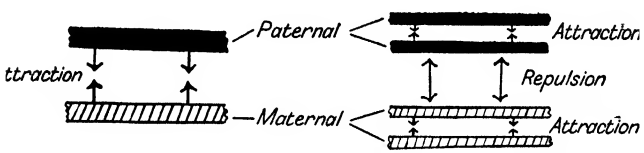


Fig. 12.—Diagrams to show how the attraction between homologous chromosomes *before* they have split becomes converted into a repulsion as soon as splitting has occurred.

mid-prophase, although it has arisen in a totally different way, by pairing of two entirely distinct chromosomes instead of by splitting of a single one. Another important point of difference is that the pachytene bivalent has two distinct centromeres, whereas the mitotic prophase chromosome has only one which does not divide until prometaphase.

Naturally the double pachytene threads are twice as thick as the single leptotene threads; they are also a good deal shorter since contraction takes place during zygotene.

Half-way through pachytene the splitting which at mitosis has occurred before the beginning of prophase takes place. Thus the division process is not suppressed in the first meiotic division, it is merely

postponed until a stage which corresponds to about half-way through prophase. Pachytene can thus be subdivided into a two-strand stage (before splitting), and a four-strand stage (after splitting).

At the beginning of pachytene the two threads are lying strictly parallel, but in some organisms they soon begin to wind round one another like the two wires of a piece of electric flex ; thus when splitting takes place it results in four threads two of which are wound round the other two.

Diplotene

The force of attraction between homologous genes seems in general to be restricted to two genes at any one level, there being no ' residual attraction '. That is to say that as soon as the pachytene chromosomes have split the attraction force between the paternal and maternal chromosomes ceases to exist, being replaced by an attraction between the two chromatids of which each chromosome is composed (Fig. 12). In this respect the pachytene chromosome resembles an ordinary mitotic chromosome and differs from a salivary gland chromosome in which as many as 256 chromomeres mutually attract one another in each transverse ' band ' (page 35). As a result of the disappearance of the attraction force between the chromosomes these begin to separate. The moment when the two homologous chromosomes begin to separate marks the transition from pachytene to diplotene. If they were to separate completely we should have another stage similar to leptotene ; actually, however, they do not do so but remain held together at certain points (called *chiasmata*). If one of those chiasmata is examined it will be seen that two out of the four chromatids at this point form an X (Fig. 9*d*). These chiasmata were seen as early as 1892 [154] and are now known to be an almost universal feature of the diplotene and later stages in all organisms (certain exceptions will be mentioned in the next

chapter). There is always (apart from very rare cases) at least one chiasma in each bivalent and there may be as many as twelve (in the long bivalent of the broad bean, *Vicia Faba* [106]). As soon as the phenomenon of genetical crossing-over was discovered it was naturally suggested that the chiasmata formed the physical basis of crossing over.

The average number of chiasmata in a bivalent is known as the *chiasma frequency*. One can speak of the chiasma frequency of a particular bivalent or of the whole chromosome set in an organism. The former varies from 1·0 to about 8·56 (in the long bivalent of the domestic chicken). The genetical significance of chiasma frequencies is dealt with in the next chapter. The appearance of bivalents with one and three chiasmata respectively is shown in Fig. 9*d* and *e*. A bivalent with a single chiasma about half-way along its length forms a four-armed structure while in the case of a bivalent with several chiasmata there are a series of loops between the chiasmata. It is important to distinguish between true chiasmata and places where one chromosome of which the bivalent is composed merely passes over or under the other. The difference between these two is shown in Fig. 9*d*; in actual material it is generally quite easy to tell them apart by careful focusing up and down.

There are two possible ways of interpreting chiasmata (Fig. 13), and for a long time it was uncertain which was correct. On the first hypothesis no breaking of chromatids has taken place before the appearance of the chiasmata, and the four threads are consequently unaltered; on this hypothesis a paternal and a maternal chromatid actually 'cross over' (in the literal sense) in such a way that on one side of the chiasma a paternal chromatid is paired with a paternal and a maternal with a maternal while on the other side a paternal is paired with a maternal, and a maternal with a paternal.

On the second hypothesis two of the four chromatids

have broken at the end of pachytene at exactly the same level and re-joined diagonally in such a way as to produce an X (Fig. 13*b*). On the first hypothesis a chiasma *may* give rise (by subsequent breaking) to a genetical cross-over; on the second hypothesis a genetical cross-over (breakage and reciprocal re-fusion) has preceded the appearance of the chiasma and given rise to it. On the second hypothesis a paternal chromatid is associated with another paternal one and a maternal with another maternal one on *either* side of the chiasma. It is now known with complete certainty that the second hypothesis is the correct one. There are a number of proofs but the simplest one is given in Fig. 13*e* and *f*. It sometimes happens that there is an unequal pair of chromosomes, one homologue containing a duplication or deletion which is not present in the other. If a single chiasma is formed between the centromeres and the inequality the result will be an " unequal bivalent ' as in Fig. 13*e* and not as in Fig. 13*f*.[176] This is quite a satisfactory proof of the second hypothesis; there are others which are even more conclusive but more difficult to understand.[28, 37, 115]

Each chiasma is thus a visible sign that a single genetical cross-over has taken place. We have now to ask what is it that causes the chromatids to break, why do a paternal one and a maternal one always break at the same level, why do never more nor less than two break at that level and, finally, why does reciprocal re-fusion always take place? Entirely satisfactory answers to all these questions cannot be given as yet, but a series of preliminary hypotheses have been put forward [33, 189]; it seems certain that the initial breakage relieves a localized strain as the chromosomes separate, but exactly how this strain arises is not yet clear. It is clear that the moment of breakage is *after* the chromosomes have split and *before* they have separated, i.e. in the very short four-strand pachytene stage.

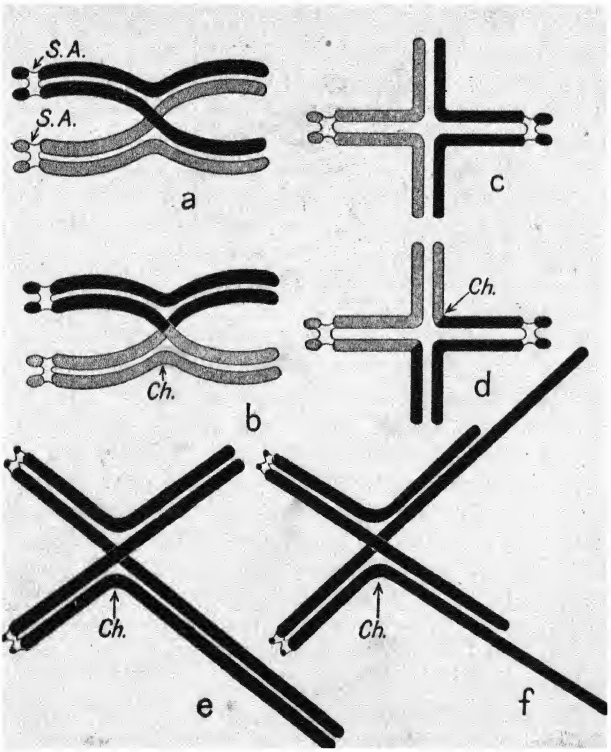


FIG. 13.—Diagrams to illustrate the difference between the old and the modern theory of chiasma-formation. In *a–d* maternal chromosome black, paternal one stippled (or vice versa). *a*—an equal bivalent with a single chiasma on the old theory; *b*—the same on the new theory, *c*—the same bivalent on the old theory after rotation, *d*—on the new theory after rotation. *e* and *f* provide the proof that the new theory is the correct one. *e* is an unequal bivalent with a single chiasma as actually found, *f* is what would happen in such a bivalent if the old theory were true (never found). S.A. = centromere.

Once the loops between the chiasmata have opened out and the diplotene bivalents have acquired their characteristic appearance (Fig. 9*d*) three kinds of changes begin to take place in the bivalents : the first two of these are universally found, while the third occurs in many organisms but not in all.

The first change is a shortening and thickening of the chromatid threads ; this can easily be seen by comparing Fig. 9*d* and *e*. It probably takes place in exactly the same way as the contraction of mitotic chromosomes between mid- and late prophase. (See Chap. II, p. 12).

The second change is most marked in bivalents with a single chiasma and consists of a relative rotation of two arms of the cross through about 180° (relative to the other two arms). The result is that a bivalent with a single chiasma which looks like Fig. 13*b* at early diplotene comes to look like Fig. 13*d* at late diplotene. In the case of bivalents with several chiasmata the rotation is usually through only about 90°—thus the successive loops between the chiasmata come to lie in planes at right-angles to one another, the alternate ones lying in the same plane, the appearance of the bivalent being similar to that of a chain stretched out tight.

The third change consists of an actual moving of the chiasmata towards the ends of the chromosomes. This is shown in Fig. 14*a*, *b* and *c*, which represent successive stages in the process. Of course the point where the paternal and maternal portions of the chromatid have fused together (the point of genetical crossing-over) does not move—all that shifts is the *visible chiasma*. This process of shifting may happen in chromosomes with one or many chiasmata ; it may be only slight or may result (as in Fig. 14*c*) in all the chiasmata moving to the extreme ends of the bivalent. In the latter case they appear never to slip off the ends, but a completely terminal chiasma has entirely lost the appearance of a cross ; it merely

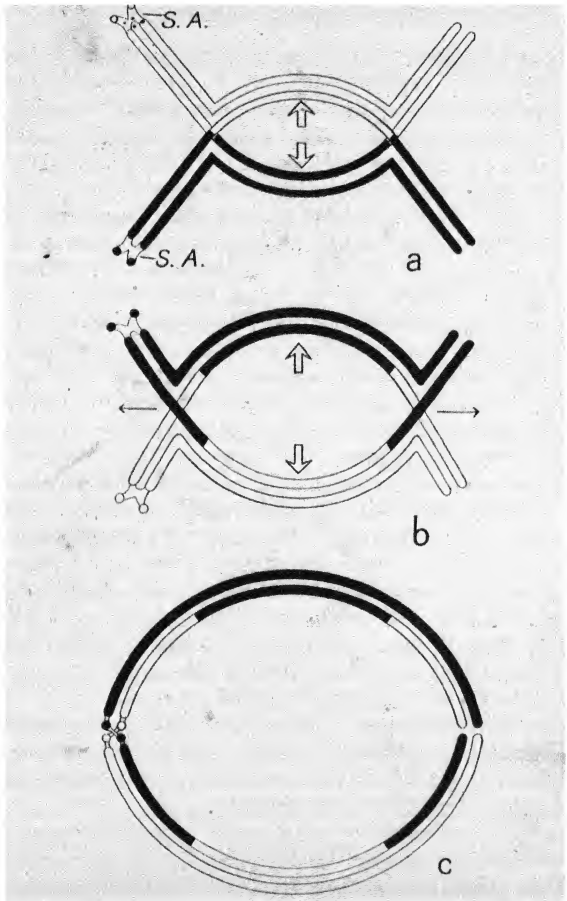


FIG. 14.—Diagrams to show terminalization of two chiasmata in a bivalent with quasi-terminal centromeres. *a*, the bivalent at early diplotene when the chiasmata correspond in position to the points of crossing-over; *b*, at late diplotene; *c*, at diakinesis when terminalization is completed. The hollow arrows represent the force of repulsion inside the loop, the solid arrows the direction in which the chiasmata are moving. S.A. = centromeres.

consists of four chromatids which are in contact, end to end (Fig. 14*c*).[31, 36]

Both the second and the third processes (' *rotation* ' and ' *terminalization* ') clearly depend in part on the general repulsion force (represented by a hollow arrow in Fig. 14). This leads in the case of rotation to the loops and ' free arms' (which may be considered as forming half-loops) taking up positions farther away from one another in space. In the case of terminalization the effect of the repulsion force will obviously be greater inside a closed loop than in a ' half-loop ' and will lead to an expansion of the loop, or loops if there are several, at the expense of the terminal half loops. Naturally as this process of terminalization takes place it involves a change in the pairing relationships of the chromatids. Whereas at first, points of crossing-over and chiasmata coincide and paternal chromatids are only paired with paternal, and maternal with maternal (Fig. 14*a*), as they diverge paternal and maternal chromatids come to lie paired for a certain distance (between the point of crossing-over and the new position of the chiasma). This shows that it is not a failure of the paternal and maternal chromatids to attract one another which prevents them remaining paired after the split has occurred in pachytene—the reason why they separate after splitting is probably merely that the two paternal threads are farther away from the two maternal ones than they are from one another.

Diakinesis

This stage corresponds to the late prophase of an ordinary mitotic division. Diplotene passes quite gradually into diakinesis so that it is quite impossible to say when one ends and the other begins. There is much to be said for abolishing the term diakinesis altogether and substituting ' late diplotene ' for it. The most noticeable difference between the bivalents in diakinesis and diplotene is that they have become

much thicker and shorter in diakinesis. As a result of the thickening of the chromatids the split between them becomes more difficult to see—in many cases it becomes quite invisible by the end of diakinesis (it will be remembered that a similar phenomenon takes place at mitosis). ' Rotation ' is usually completed by the beginning of diakinesis but ' terminalization ' may continue right up to the metaphase of the first meiotic division.

As in the late prophase of mitosis there is in diakinesis a tendency for the thickened chromosomes to move to the periphery of the nucleus and to arrange themselves on the inside of the nuclear membrane (yet another result of the general surface repulsion force). This tendency is only seen, however, when the volume of the nucleus is very much greater than the volume of the bivalents (as it is, for example, in the spermatocytes of the Pulmonata, but not in those of the Orthoptera).

Prometaphase

As in the case of mitosis we call the period between the disappearance of the nuclear membrane and the moment when the spindle is fully formed, prometaphase. At this stage the diakinesis bivalents have contracted still further and begin to be associated with the developing spindle. It will be remembered that at mitosis the contraction of the chromatids is due to the actual substance of which they are composed becoming ' spiralized ' ; that is to say each prometaphase or metaphase chromatid at mitosis consists of a cylindrical ' spring ', possibly enclosed in some sort of sheath or pellicle. By special technical methods (exposure of the living material to ammonia vapour or fumes of strong acids before fixation) it has been shown [96, 98] that at meiosis each chromatid is not merely a spiral but a *double spiral* (Fig. 15), each gyre of the ' major ' spiral being made up of several gyres of the ' minor ' spiral. This may

also be the case at mitosis, but so far only one spiral has been observed at ordinary somatic divisions.

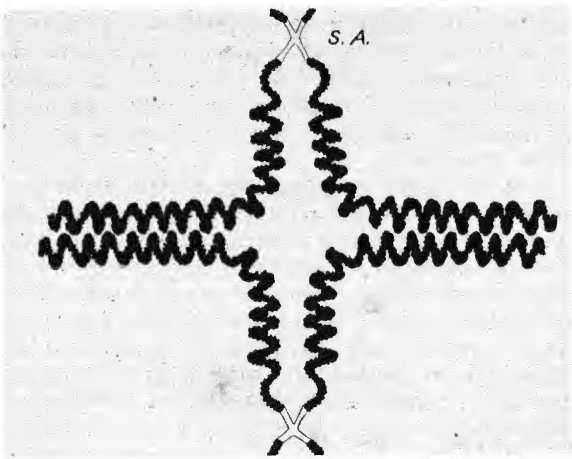


FIG. 15.—Diagram of a bivalent at the first meiotic division showing the major and the minor spiral. The centromeres (S.A.) are subterminal and there is only one chiasma.

METAPHASE OF FIRST MEIOTIC DIVISION

At mitosis each chromosome is attached to the spindle by a single centromere (or one which has only just divided in prometaphase and whose component halves are still in contact). As a result all the centromeres lie in the equatorial plane. At meiosis, on the other hand, each bivalent possesses two centromeres, one belonging to the maternal chromosome, the other to the paternal one. These are in general fairly far apart and they arrange themselves at equal distances above and below the equatorial plane, i.e. between this and the poles of the spindle. *The centromeres have not divided, nor do they do so until the second meiotic division.*

Anaphase of First Meiotic Division

At the anaphase of the first division the whole centromeres play the same rôle as the split halves of the centromeres play at an ordinary mitotic anaphase. As they separate they drag after them the chromatids which are attached to them. The chiasmata which have not already been ' terminalized ' move along to the ends of the bivalent away from the centromere and slip off at the end. Chromosomes which are only associated by means of ' terminal chiasmata ' are simply torn apart. Each centromere as it moves towards the pole drags after it (either on the surface of the spindle or in its substance) two chromatids. If the centromere is median or submedian these will form a structure with four arms of more or less equal length with the centromere at their point of junction (as in the case of the larger chromosome in Fig. 10*h* and chromosome A in Fig. 16). If, on the other hand, the centromere is sub-terminal, two of the arms will be very short and the general appearance of the chromosome will be that of a V (smaller chromosome in Fig. 10*h* and chromosomes B and C in Fig. 16).

The result of the first meiotic anaphase is often said to be a separation of whole chromosomes instead of the split halves of chromosomes as at mitosis. While this is correct in a sense it must be pointed out that the chromosomes which separate at the first anaphase are not the same as the maternal and paternal chromosomes which came together at zygotene ; these have interchanged sections of their length by crossing over so that the actual chromosomes which separate at the first division are *new* combinations of segments of the maternal and paternal chromosomes (Fig. 16). Between the centromere and the first point of crossing over on either side of it, however, the first anaphase always leads to the separation of two maternal from two paternal chromatids.

Whether the maternal centromere in a particular

bivalent goes to the 'North' or the 'South' pole of the spindle (and vice versa for the paternal one) is a matter of chance; it depends on which way up the bivalent has orientated itself at prometaphase. There is no correlation between the mode of orientation of one bivalent and another in the same cell. Thus in *Drosophila melanogaster* with four bivalents

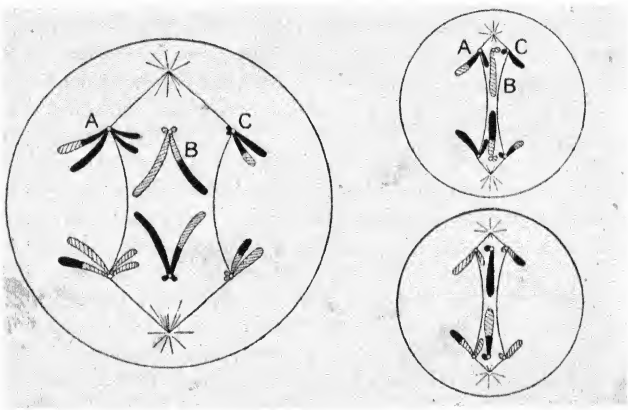


FIG. 16.—Diagrams showing the genetic consequences of the first and second meiotic divisions. Maternal portions black, paternal ones hatched. Three pairs of chromosomes are shown, each pair having possessed a single chiasma. It will be seen that the first division is always 'reductional' between the centromere and the first chiasma, and the second division is always 'equational' for this region.

all paternal centromeres will go to the same pole once in 16 (2^4) times, in female Locusts and grasshoppers with 12 bivalents once in 4,096 (2^{12}); while in man with 24 bivalents this event will only happen once in 16,776,116 (2^{24}) times. Exceptions to this rule occur under special circumstances in *Sciara* and *Oenothera*.

As in the case of mitosis the first part of anaphase (during which the centromeres repel one another) is followed by a period during which the middle part of

the spindle elongates so as to form a ' stem-body ' and completes the anaphase separation of the two groups of chromosomes.

Telophase

The telophase of the first meiotic division does not differ in any important respect from that of an ordinary somatic mitosis. The two telophase nuclei may pass into a more or less complete resting stage between the two meiotic divisions (*interphase* or *interkinesis*) in which the chromosomes become unfixable as in a somatic resting stage or they may remain condensed and undergo no changes between the anaphase of the first division and the metaphase of the second (Fig. 10*i*).

Second Meiotic Division

If the chromosomes have not gone into a resting stage during interkinesis (a matter which is subject to a surprising amount of variation even in the same organism under different conditions) there will naturally be no prophase to the second division. In this case the telophase nuclei of the first division will pass directly (by loss of their nuclear membranes) into the prometaphase of the second division ; a spindle will develop and we have reached the metaphase of the second division by a ' short-cut ', involving the entire elimination of a prophase. In any case the prophase of the second division, even if present, is always short.

The prometaphase of the second division differs from that of an ordinary mitosis in two respects, (1) the number of chromosomes is half the somatic number, (2) the chromatids diverge widely, being only held together at the centromere and not approximated throughout their length as at mitosis ; they tend, however, to come into closer contact at the metaphase of the second division. The second division chromosomes appear to resemble those of an

ordinary mitosis in having only one spiral (and not a 'major' and a 'minor' one as at the first meiotic division).

Anaphase and Telophase of the Second Division

These do not differ from those of an ordinary mitosis, so that it is not necessary to describe them in detail. Between the centromere and the first point of crossing-over the second anaphase always leads to the separation of maternal from maternal chromatids or of paternal from paternal ones (Fig. 16).

One last question in connexion with meiosis remains to be considered. If the whole process has arisen by profound modification of two mitoses, and if the chromosome 'split' of the first one is delayed until pachytene, what has happened to the 'split' corresponding to the second division? Usually there is no trace of it but various authors [97, 170] have claimed to have seen a split down the middle of the anaphase chromatids of the first division. Such a split, if present, either closes up again, and is thus non-functional, or else functions as the effective split at the next division (in animals the first cleavage division of the zygote, in Angiosperms the pollen grain mitosis in microsporogenesis and the first division of the embryo-sac nucleus in megasporogenesis). The univalent chromosomes of certain hybrids actually split in both divisions, so that the 'potential' split may become functional under exceptional circumstances.

CHAPTER V

SPECIAL PROBLEMS OF MEIOSIS

WE have seen in the preceding chapter that crossing-over is an event which normally happens at least once in every pair of chromosomes at meiosis. If a bivalent has a chiasma-frequency of 1·0 that means that on an average two out of the four chromatids undergo one cross-over, i.e. that between two points situated at opposite ends of the chromosome, crossing-over takes place in 50 per cent of cases. Thus a bivalent with a chiasma-frequency of 1·0 will have a length of 50 'genetic units'; similarly, bivalents with chiasma-frequencies of 2·0 and 3·48 will have map lengths of 100 and 174 units respectively. In mapping chromosomes genetically it is useful to know the total map-length in advance, and this can now be calculated from the cytologically determined chiasma-frequency in this simple way. Table V shows the total calculated length of the genetic maps in maize and the length already known. It will be seen in no case does the known length exceed the total calculated length.

In some chromosomes it appears that chiasmata are as likely to be formed in one region as in another, so that if we divide the bivalent into n short lengths x microns long the chiasma-frequency of all of them will be the same. This appears to be the case in the long chromosomes of *Vicia faba*,[106] *Lilium* spp.,[116] *Stenobothrus* and *Chorthippus*,[30, 40] although it is probable that even in these instances the distribution of chiasmata is not quite at random. In certain organisms, however, chiasmata are more or less restricted to definite regions. Thus in the female *

* In *Drosophila* genetic maps only exist for the female sex, since no crossing-over normally takes place in the male (*vide infra*).

Drosophila melanogaster an examination of the genetic map reveals the fact that the genes in Chromosomes II and III are crowded in the central region round the centromere ; there is also a lesser degree of crowding at the ends, the genes between

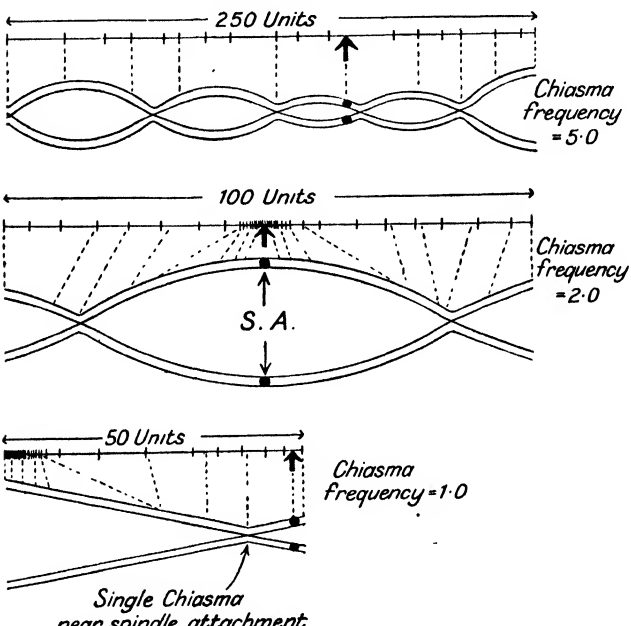


FIG. 17.—Diagrams of the relationship between chiasma-localization and genetic maps. Explanation in text.

the ends and the central region being much more sparsely scattered (Fig. 17). By a study of chromosome breaks it has been possible to show that this is a 'genetical illusion'—in the actual physical chromosome the genes are approximately evenly spaced. The central region is shortened on the genetic map

while the part between this and the ends is artificially magnified. This means that crossing-over takes place more often in this region than in a region of the same length near the centromere; there is a *partial localization* of chiasmata.

In a number of organisms—*Fritillaria* (Liliaceae), *Mecostethus* (Orthoptera, Acrididae) and many Tettigonidae—another type of localization is found in which chiasmata are entirely restricted to certain regions of the chromosomes (usually the region next to the centromere). Thus in the grasshoppers of the genus *Mecostethus* there is usually a single chiasma in each bivalent which is situated very close to the centromere.[181] In this, which is an extreme case, only those genes which lie near the centromere could

TABLE V

CHIASMA-FREQUENCIES AND GENETIC LENGTH OF THE TEN CHROMOSOMES IN MAIZE (*ZEA MAYS*) [36]

Chromosome	Chiasma frequency	Map-length in genetic units		Number of known genes
		Total calculated	Already known	
1	3·65	183	128	7
2	3·25	163	71	7
3	3·00	150	103	7
4	2·95	148	111	10
5	2·95	148	72	6
6	2·20	110	61	5
7	2·45	123	52	7
8	2·45	123	18	2
9	2·20	110	70	7
10	1·95	98	32	3
Totals	27·05	1,353	618	60

be 'mapped' genetically; the others would show complete linkage and no crossing-over (Fig. 17).

It is a fact which has long been known in genetics

that the occurrence of a cross-over at a particular locus prevents crossing-over for a certain distance on either side. This phenomenon is known as *interference.* Cytologically it means that there must be a certain minimum distance between chiasmata. If, as previously suggested, crossing-over results from a local strain of some kind it is clear that this strain will be relieved in the adjoining region once crossing-over has taken place. That is to say, a chiasma diminishes the probability of another being formed in its immediate neighbourhood to zero and this ' interference ' falls off until at a certain distance from the first chiasma it is no longer present. In many small bivalents the total length of the chromosome is shorter than the region of complete interference so that only one chiasma is formed. HALDANE [61] has proved the existence of interference as a cytological phenomenon by a statistical analysis of chiasma-frequencies.

If one considers two adjacent chiasmata in a bivalent it is clear that the relationship between them may be of three different kinds : (1) the second chiasma may involve the *same two chromatids* as the first one, (2) it may involve one of those which crossed over in the first chiasma and one that did not, or (3) it may involve the two that did not cross over in the first chiasma. These three types of relationship are called *reciprocal, diagonal* and *complementary.*[158] If the second chiasma arose between the four chromatids at random then the three types would occur in the ratio 1 : 2 : 1. The reciprocal and complementary types of relationship cannot usually be distinguished from one another cytologically ; they are grouped together as *compensating* relationships.

MEIOSIS IN HYBRIDS

From the cytological point of view there are three main types of hybrids, ordinary diploid hybrids,

complex heterozygotes (such as the Evening Primrose, *Oenothera* spp.) and polyploid hybrids (allopolyploids). A diploid hybrid is an organism with its two haploid sets of chromosomes derived from different parent species. However similar they may be taxonomically these parent species will always differ in respect of a number of genes; moreover, the extent of the taxonomic differences between two species is probably a very unreliable guide to the number of gene differences involved.

In addition to simple gene-differences the parents of a hybrid may also differ in the way in which the genes are arranged in the chromosomes. Thus in the case of *Drosophila melanogaster* and *D. simulans* (which are taxonomically so similar that they were originally regarded as one species), the number of chromosomes is the same and their relative lengths are almost identical, but a large portion of the IIIrd chromosome (about one-quarter of its total length) is inverted in *simulans* in comparison with *melanogaster*; in addition there are a number of other differences in gene-sequence which are visible in the salivary gland nuclei.[66, 145] Hybrids can be obtained between the two species, but they are sterile.

Inversions of the type just described are probably among the commonest cytological differences between closely related species (they occur also inside single species.[37, 46]) In addition to these and single gene-differences, however, the parent species of a hybrid may differ in respect of (1) chromosome number, (2) chromosome size, and (3) rate and extent of nucleination of the chromosomes at mitosis and meiosis. Where the chromosome numbers of the two parent species differ one of the two haploid sets in the hybrid will contain more chromosomes than the other and the 'extra' chromosomes will usually form univalents at the first meiotic division, although in some instances two chromosomes of one species may pair with one of the other species, so that a trivalent is

formed in the hybrid. As far as the others are concerned, there is a possibility of them all pairing and forming bivalents, but where the chromosome numbers of the two species differ the degree of homology between the two haploid sets is usually so incomplete that pairing and chiasma-formation only takes place in a few chromosomes ; the number of univalents is thus usually considerably in excess of the number of ' extra ' chromosomes in such cases. For example in hybrids between the moths *Saturnia pavonia* and *S. pyri* (haploid numbers 29 and 30 respectively) only about 5 to 6 bivalents are formed as a rule, the remaining 47 or 49 chromosomes being left as univalents.[144]

Where the chromosome numbers of the two parent species are the same *all* the chromosomes *may* pair but do not necessarily do so. Thus in the hybrids between the moths *Celerio euphorbiae* and *C. galii* (both with a haploid number of 29) each *euphorbiae* chromosome pairs with a *galii* one so that 29 bivalents and no univalents are formed. On the other hand, in the hybrid between *Pergesa elpenor* and *Celerio euphorbiae* (each with a haploid number of 29 as before) only about 4 bivalents are formed, the remaining 50 chromosomes being left as univalents.[16] Finally, in diploid hybrids between the Cabbage and Radish no bivalents may occur, all the chromosomes forming univalents.[81, 82]

Univalent Chromosomes at Meiosis [153, 41, 83]

A univalent chromosome at meiosis may arise in two ways—it is either a chromosome which has never undergone pairing at zygotene, or else it is one which has paired to form a bivalent whose two component chromosomes have separated again at diplotene owing to the fact that no chiasma was formed between them. Most univalents are probably of the former type, but only a close study of all stages of meiosis can reveal the precise mode of origin in

each particular case. The behaviour of univalents at the metaphase of the first meiotic division is interesting, although variable. Since they only possess one undivided centromere (instead of two as in the case of a bivalent, or one which is in process of division as in a mitotic chromosome) they do not necessarily become associated with the spindle in the equatorial plane, but attach themselves anywhere between its two poles. As the bivalents separate into their component halves and pass to the poles the univalents are left in the central part of the spindle (the ' stem-body '). The first meiotic division may be completed without any separation of the univalents into their chromatids in which case they may (1) be distributed at random between the two nuclei, (2) form small supplementary nuclei enclosed in their own nuclear membranes, (3) mechanically prevent complete separation of the two main groups of chromosomes after the first division so that these come to be included in a single *diploid* interkinesis nucleus. Alternatively, (4) the centromeres of the univalents may finally undergo division at the end of the anaphase of the first division, so that the half-univalents pass to the poles although tardily. In the first three cases (where the centromeres of the univalents have not divided in the course of the first division) the univalents behave normally at the second division, separating into their component chromatid halves. In the fourth case the centromeres have divided and the univalents separated into their chromatid halves at the first division, and consequently do not divide at all at the second meiotic division, being passively distributed at random to the two poles.

Meiosis in the Male *Drosophila* and in other Diptera

It is well known that in the males of *Drosophila* spp. no crossing-over normally takes place. The

reason for this has been cleared up by a recent investigation.[32] No chiasmata are normally formed in the autosomes ; if this were to take place in any other organism but a Dipteran it would lead to the bivalents separating into pairs of univalent chromosomes at diplotene, but owing to the strong ' somatic pairing ' (residual attraction) the two chromosomes of each bivalent do not fall apart, but remain associated until anaphase. In the case of the X and Y chromosomes it is possible that a pair of compensating chiasmata are formed in the neighbourhood of the centromere in a region which is inert and ' homologous '. The normal process of meiosis in the male *Drosophila* is thus highly modified, although in the female it follows the usual course. All the ' higher ' Diptera (Brachycera) seem to possess the same type of meiosis as *Drosophila*, lacking chiasmata in the male ; but in some of the Nematocera (Culicidae, Chironomiidae and Tipulidae at any rate) there is a perfectly normal meiosis in the males.

In other Diptera such as species of the genus *Sciara* even more unusual types of meiosis are found in the males, although, as in *Drosophila*, oögenesis is entirely normal. Thus in the male diploid set of *Sciara coprophila* there are five pairs of chromosomes (one pair of large V's, one pair of medium-sized V's and three pairs of rods). There is no pairing of chromosomes at zygotene and all ten behave as univalents. The spindle which forms at the prometaphase of the first meiotic division is a half-spindle (see Fig. 3) to which *both* the large V-shaped chromosomes and one member of each of the other pairs are attached. The remaining chromosomes move away from the half-spindle ; there is genetic evidence that these four chromosomes which are unattached to the half-spindle are all paternal chromosomes. The first meiotic division thus separates a group of six chromosomes from a group of four—the latter degenerate in a small bud of cytoplasm which becomes

cut off from the main cell like a polar body, while the group of six chromosomes proceed to the second meiotic division. Here a normal bipolar spindle is formed and five of the six chromosomes divide normally, but one of the rod-shaped chromosomes divides in such a way that both its halves go to the same pole. The group of chromosomes at this pole form a sperm nucleus, while the other group degenerates. Only one sperm is thus formed from each primary spermatocyte and it contains more than the haploid number of chromosomes.[125] A complicated mechanism involving the elimination of certain chromosomes occurs during the cleavage divisions.[47] It is clear that this type of meiosis is even more highly modified from the normal type than that found in *Drosophila*, and that it involves a complete suppression of crossing-over in the male. In the female *Sciara*, on the other hand, meiosis is entirely normal. Some of the Cecidomiidae (Gall-midges) have an extraordinary chromosome cycle, the germ-line being polyploid in both sexes, although the soma is diploid. An elimination of whole chromosomes takes place during the cleavage divisions of those nuclei which will give rise to the somatic tissues of the adult. Spermatogenesis is highly anomalous, but the details have not been fully worked out yet. In the Hessian Fly (*Phytophaga destructor*) the germ-line seems to be tetraploid,[120] while in *Miastor metraloas* it is probably octoploid.[95]

Genetically Determined Abnormalities of Meiosis

A fairly large number of gene-mutations are now known which lead to abnormalities of meiosis. Most of these mutations interfere with chiasma-formation, and consequently lead to very irregular distribution of the chromosomes at the first meiotic division. Such genes are known in Maize, *Nicotiana* and other

plants. In *Drosophila melanogaster* 'Gowen's Gene' produces similar effects on meiosis in the female.[55]

Behaviour of Sex-Chromosomes at Meiosis

We have briefly considered (Chap. III) the different types of sex-chromosomes as they appear at mitosis; it remains to be seen what happens to them at meiosis. In the homogametic sex they behave, in general, just like autosomes. Their behaviour in the heterogametic sex depends on the following general principles:

(1) Non-homologous regions of chromosomes do not pair, and consequently no chiasmata can be formed between them.
(2) Homologous regions do pair at zygotene, even though other regions of the chromosomes in question are not homologous. Chiasmata will be formed in the homologous paired regions, unless these are very short.
(3) Pairing may be prevented, even between homologous regions, by extreme positive heteropycnosis.
(4) Univalent sex-chromosomes (i.e. sex-chromosomes with no chiasmata) behave like other univalent chromosomes and may show either the first or the fourth type of behaviour described above (p. 73).

Having stated these general principles we can consider specific cases. In Locusts and Grasshoppers we encounter one of the most highly evolved types of sex-determining mechanism, which is, however, one of the simplest to understand (see *Frontispiece*, Fig. 3). The male is the heterogametic sex, and there is no Y-chromosome; (that is to say the female diploid set includes two X-chromosomes, the male set only one, which is consequently a univalent at meiosis). The X in the male shows intense positive hetero-

pycnosis in its distal half (i.e. that farthest away from the centromere which is almost terminal) throughout zygotene and pachytene. This heteropycnosis extends to the proximal half during diakinesis and prometaphase, after which de-condensation usually begins to set in. The X-chromosome associates itself with the spindle of the first meiotic division somewhere between the equator and one of the poles. Its centromere does not divide at the first division and consequently both the chromatid halves of the X go to the same pole. Thus two kinds of secondary spermatocytes are formed, one with, the other without an X-chromosome. At the second division the X divides and goes to both poles in those secondary spermatocytes in which it is present. Thus in this case the univalent X shows the first type of behaviour of the four mentioned above. The opposite condition where a single X divides in the first meiotic division and goes undivided to one pole in the second division is met with in some other insects (the Beetle *Photinus* and the whole of the Hemiptera Heteroptera, with one or two exceptions.[169, 192, 150, 163] The Locust *Schistocerca* and the Bug *Archimerus* also illustrate the truth of the third principle (that extreme positive heteropycnosis may prevent either pairing or chiasma-formation or both) since even in polyploid spermatocytes containing two or three X-chromosomes these behave as univalents, although they lie very close to one another, as a result of an attraction analogous to 'somatic pairing'[178, 194] (Fig. 18).

A more complicated type of sex-determining mechanism is found in mammals, where both an X and a Y are present in the male. In the Rat[94] the two sex-chromosomes both have submedian centromeres. There is a region in the Y which is homologous to a corresponding region in the X. This region includes the centromeres in both chromosomes and extends as far as one end of the chromosome. The remaining parts of the two chromosomes are not

homologous. Thus each sex-chromosome consists of two segments, a *pairing segment* and a *differential segment* (Fig. 8). The two pairing segments come together at zygotene and chiasmata are later formed between them on either side of the centromeres or on both sides. An *XY bivalent* is thus formed. At the first meiotic division the differential segments of the X and Y go to opposite poles and at the second division they divide in the ordinary way. The sex-determining mechanism in man is probably of this type but the details have not yet been worked out. In some Marsupials the sex-chromosomes are essentially of the same kind as in the Rat, but the centromeres appear to lie in the differential region.[93]

In *Drosophila melanogaster* we again have an XY bivalent formed in the male; the pairing segment includes the centromere and is genetically inert. The X-chromosome has a single differential segment which includes the centromere and contains all the 'sex-linked' genes except *bobbed*, while the Y has two differential segments, one on either side of the pairing segment.

In the Hemiptera Heteroptera no pairing of the X- and Y-chromosomes takes place at the first division. There is thus probably no true pairing segment common to both X and Y. The two chromosomes are quite separate at the first meiotic division, and they both divide 'equationally' so that the two secondary spermatocytes which are formed have the same chromosome set. At the second meiotic division the X- and Y-chromosomes come into extremely close approximation. They orientate themselves in the long axis of the second division spindle, above and below the equatorial plane, so that one goes to each pole (cf. *Anasa*, *Alydus* and *Protenor*, where there is a similar mechanism but without a Y). The Coreid Heteropteran *Archimerus* (*Frontispiece*, Fig. 4) is exceptional in that its X-chromosome divides in the second instead of in the first meiotic division. Thus in

this XO Bug two kinds of secondary spermatocytes are formed (plus-X and minus-X) instead of only one, as in most Heteroptera.

In some organisms with an X_1X_2Y mechanism (e.g. the grasshopper *Paratylotropidia* [89] and certain Praying Mantids) each X has a pairing region homologous to a corresponding pairing region in the Y. No region in X_1 is homologous to any part of X_2, so that X_1 and X_2 never pair together but always with different parts of the Y.[88, 184, 187] The converse situation occurs in *Rumex acetosa* where Y_1 and Y_2 pair separately with parts of the X.[159] Thus in both cases a *sex-trivalent* is formed with its three component chromosomes held together by chiasmata. In the Mantids X_1 and X_2 go to the same pole at the first division, Y going to the other pole, in *Rumex acetosa* Y_1 and Y_2 go to one pole and X to the other.

Meiosis in Haploid Organisms

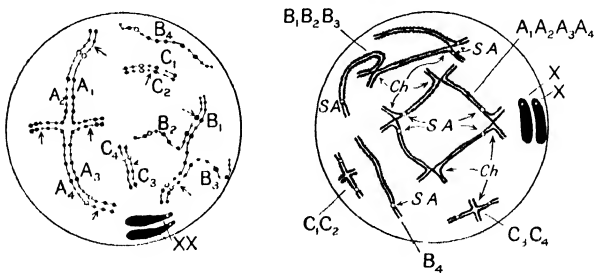
In Insects with male haploidy it is usual for the first meiotic division to be entirely suppressed so that the sperms are formed with the same number of chromosomes as the somatic tissues of the adult male. Vestiges of the first meiotic division do occur, however, in some cases. Thus in the male Bee (drone) the prophase stages of the first meiotic division take place, but the metaphase and anaphase are entirely omitted ; the second meiotic division then follows [134] ; in some other haploid insects the first division is completely suppressed.[69] In plants, on the other hand, where haploid sporophytes occur occasionally, both meiotic divisions usually occur. In 'true' haploids all the chromosomes behave as univalents, dividing in either the first or the second division, but never in both. Some haploid plants, however, have small sections of the chromosomes reduplicated in other members of the chromosome set ; in these cases the small reduplicated sections may pair at zygotene and form chiasmata later, so that there are a few

bivalents at the first meiotic division.[21] Plants like this are clearly not true haploids; they are really intermediate between the diploid and the true haploid condition.

Meiosis in Polyploid Organisms

Where there are more than two homologous chromosomes of each kind in the somatic set three or more may become paired at zygotene—but never more than two at any one point. Thus in a triploid if we consider three homologous chromosomes A_1, A_2 and A_3, A_1 may pair with A_2 in one region and with A_3 in another, but A_1, A_2 and A_3 are never associated together in the same region.[138] On the other hand, A_1 may pair with A_2 throughout its entire length (to form a bivalent) in which case A_3 will be left unpaired and form a univalent. Thus in a triploid organism *trivalents*, bivalents, and univalents may be found in the same nucleus. Similarly, in a tetraploid *quadrivalents* may be found in addition to the three other types and in higher polyploids *quinquevalents* and *hexavalents* may also occur. All associations of more than two chromosomes can be spoken of collectively as *multivalents*. The frequency of formation of multivalents in polyploids varies a great deal and apparently depends in part on the length of the chromosomes and in part on the rapidity of zygotene pairing.[178] Where the chromosomes are long and pairing is slow the probability of multivalent formation is high, where the chromosomes are short and pairing is rapid it will be low. Other factors such as the ratio of the volume of the chromosomes to the volume of the nucleus and the arrangement of the chromosomes at zygotene (random or polarized—see Chap. IV) may also affect the frequency of multivalent formation. Another factor of major importance in this connexion is whether the polyploid in question is an auto- or an allo-polyploid; allopolyploids form far fewer multivalents than auto-

polyploids. Thus if we consider an allotetraploid with four chromosomes A_1, A_2, A_3' and A_4' (A_1 and A_2 being derived from one parent species and A_3' and A_4' from the other) it will probably form two bivalents (A_1, A_2) and (A_3', A_4') since although all the chromosomes are partly homologous those derived from the same parent are completely so. An idea of the range of variation as regards multivalent



a Two-Strand Pachytene. b Diplotene.

FIG. 18.—Diagrams of meiosis in a male tetraploid organism which has a somatic chromosome set of twelve autosomes and two X-chromosomes. One quadrivalent ($A_1A_2A_3A_4$) one trivalent ($B_1B_2B_3$), two bivalents (C_1C_2 and C_3C_4), and a univalent (B_4) are formed among the autosomes. The two X-chromosomes are held together in close association as a result of a pairing-attraction, but do not form any chiasmata on account of their strong positive heteropycnosis. In *a* the positions where the chiasmata will subsequently arise are indicated by arrows. S.A. = centromere.

formation can be got from Table VI which shows the frequency with which quadrivalents are formed in autotetraploids.

The general history of multivalents at meiosis (chiasma-formation, shifting of chiasmata, &c.) follows the usual course already described in the case of bivalents. When they come to orientate themselves on the spindle of the first division they do so in a way which can be explained if there is, in

addition to the general repulsion force between all chromosome surfaces, an extra repulsion force between centromeres. Thus in the case of a trivalent it is usual for two centromeres to go to one pole and the one between them to the other. In the case of

TABLE VI

FREQUENCY OF QUADRIVALENT-FORMATION IN AUTOTETRAPLOIDS

Organism	Maximum possible no. of Quadrivalents	Actual no. (average)
Artemia salina	21	0·0
Tulipa stellata	12	0·5
Schistocerca gregaria . .	11	3·0
Primula sinensis . . .	12	10·4
Datura Stramonium . .	12	12·0

quadrivalents two centromeres go to each pole. Which of the four go to the same pole depends in part on whether they lie in the middle of the chromosomes or almost at one end and in part on the number of chiasmata which have been formed in the quadrivalent. In nuclei with only quadrivalents and bivalents an equal number of chromosomes will go to the two poles at the first division, but in those with univalents, trivalents or quinquevalents the number of chromosomes going to the poles will generally be unequal.

MEIOSIS IN COMPLEX HETEROZYGOTE ORGANISMS [29, 35, 21]

In many species of the genus *Oenothera* (Evening Primroses) and in some other plants such as *Hypericum punctatum* [65] and *Rhoeo discolor* [91] it is not possible to arrange the somatic chromosomes in pairs ; that is to say that no one chromosome is completely homologous to any other. Only as a result of long

genetical and cytological work has it been possible to understand exactly what happens during the meiosis of such organisms. The following account is necessarily somewhat simplified but is substantially true.

If the chromosomes of an ordinary diploid organism with a haploid number of 4 be represented as follows :

abcdef	*ghij*
abcdef	*ghij*
klmno	*pqrst*
klmno	*pqrst*

then those of *Oenothera muricata* will be :

$ab\mathrm{C}_1cd$	$lk\mathrm{R}_3mn$	$vu\mathrm{C}_6wx$
$dc\mathrm{R}_1ef$	$nm\mathrm{C}_4op$	$xw\mathrm{R}_6yz$
$fe\mathrm{C}_2gh$	$po\mathrm{R}_4qr$	$zy\mathrm{C}_7\alpha\beta$
$hg\mathrm{R}_2ij$	$rq\mathrm{C}_5st$	$\beta\alpha\mathrm{R}_7ab$
$ji\mathrm{C}_3kl$	$ts\mathrm{R}_5uv$	

It will be seen that each chromosome consists of three segments, two *terminal ones* (represented by the small letters) and a *median one* (represented by the capital C's and R's). The median segment contains the centromere in all cases. None of the median segments are homologous (i.e. they are *differential* segments analogous to those present in sex-chromosomes) ; the terminal segments in each chromosome are, however, homologous to two other terminal segments *in different* chromosomes. Thus when pairing takes place at zygotene it does not generally affect the median segments * but only the terminal ones (Fig. 19*a*). At pachytene a continuous ring of chromosomes is formed instead of a number of bivalents. Chiasmata arise in the paired terminal segments so that the ring is maintained up to the metaphase of the first meiotic

* The median segments are sometimes partially homologous, in which case they may pair and form occasional chiasmata (see Chap. VI).

division. Occasionally a chiasma fails to develop in one of the paired terminal regions, so that the ring breaks at one point to give an open chain. It is obvious from the homologies of the pairing regions that the relative positions of the 14 chromosomes in the ring is constant.

At the prometaphase of the first division the centromeres arrange themselves in a zig-zag round the equator of the spindle so that alternate ones go to the same pole at anaphase (Fig. 19). This is a natural consequence of the repulsion between the centromeres, and is a phenomenon we have already met in the case of trivalents. It results in all the C median segments going to one pole and the R ones to the other. C_1 . . . C_7 and R_1 . . . R_7 are thus inherited as units and are referred to in the genetical terminology as the *curvans* and *rigens complexes*. *Oenothera muricata* thus consists of a rigens complex, a curvans complex and 14 pairing segments each of which is represented twice in the somatic chromosome set.

The process of meiosis is essentially the same in macro- and micro-sporogenesis so that two kinds of megaspores and two kinds of pollen grains are formed. One of these contains the curvans segments C_1–C_7 and the other the rigens segments R_1–R_7. Fertilization of a curvans ovule by a curvans pollen tube or a rigens ovule by a rigens pollen tube leads, however, to an inviable type of zygote. Thus only the curvans × rigens and rigens × curvans zygotes survive and the species is kept in a state of permanent heterozygosity as far as the middle parts of its chromosomes are concerned.

The condition described above, in which all the chromosomes form a ring is found in a number of species of Oenothera, but not in all. In the remainder intermediate stages between this condition and normal homozygosity are found. Thus in *Oenothera lamarckiana* a ring of twelve chromosomes and a

single bivalent are formed at meiosis, in *Oe. biennis* two rings (of eight and six chromosomes respectively) and in *Oe. franciscana* a ring of four and five bivalents ;

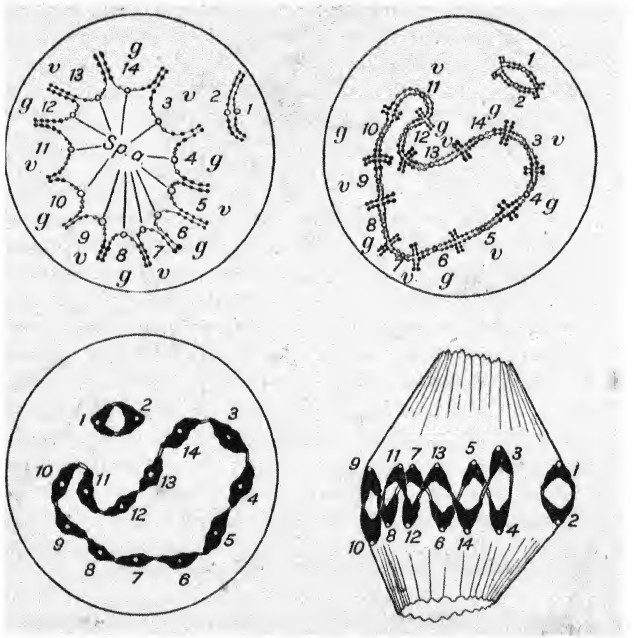


FIG. 19.—Diagrams of the First Meiotic Division in *Oenothera lamarckiana*. The chromosomes are labelled 1–14. 1 and 2 form a bivalent, being homologous throughout. 3–14 form a ring of 12 chromosomes united by 12 chiasmata after diplotene. The middle segments containing the *velans* and *gaudens* genes are labelled *v* and *g*. These segments contain the median centromeres. At anaphase chromosomes 3, 5, 7, 9, 11 and 13 go to one pole, 4, 6, 8, 10, 12 and 14 to the other.

Oe. Hooker is completely homozygous and forms seven bivalents. We can represent the chromosomes of the first two species as follows : (the ' complexes ' are

called *velans* and *gaudens* in *Oe. lamarckiana*, *rubens* and *albicans* in *Oe. biennis*):

Oe. lamarckiana		*Oe. biennis*	
abcd	$po\mathrm{G}_3qr$	$ba\mathrm{R}_1cd$	$po\mathrm{A}_4qr$
abcd	$rq\mathrm{V}_4st$	$dc\mathrm{A}_1ef$	$rq\mathrm{R}_1st$
$fe\mathrm{V}_1gh$	$ts\mathrm{G}_4uv$	$ef\mathrm{R}_2gh$	$ts\mathrm{A}_1uv$
$hg\mathrm{G}_1j_4$	$vu\mathrm{V}_5wx$	$hg\mathrm{A}_2ij$	$vu\mathrm{R}_6wx$
$ji\mathrm{V}_2kl$	$xw\mathrm{G}_5yz$	$ji\mathrm{R}_3kl$	$xw\mathrm{A}_6yz$
$lk\mathrm{G}_2mn$	$zy\mathrm{V}_6\alpha\beta$	$lk\mathrm{A}_3ab$	$zy\mathrm{R}_7\alpha\beta$
$nm\mathrm{V}_3op$	$\beta\alpha\mathrm{G}_6ef$	$nm\mathrm{R}_4qp$	$\beta\alpha\mathrm{A}_7mn$

Rings of four, six or more chromosomes are formed at the first meiotic division in a number of other plant genera (*Campanula*, *Pisum*, &c.) and from a study of these cases (some of which, notably *Pisum*, have arisen under experimental conditions) it has been possible to explain the origin of the whole mechanism (Chap. VI).

CHAPTER VI

CHROMOSOMES AND EVOLUTION

THE process of evolution includes both morphological change and the origin of new species. In the course of time a species may undergo changes both structural and physiological, without giving rise to any new species. On the other hand the formation of a new subspecies or species always involves some degree of morphological change, however slight. Modern authors have found it convenient to use the term *speciation* to describe the processes whereby new species come into existence, reserving the term evolution for all types of secular biological change.

There have been many attempts to define species. It is now clear that it is not possible to adopt any

purely morphological definition that will apply to all groups of organisms. The nature and degree of taxonomic characters which are regarded as valid specific differences vary from group to group. Nevertheless the species remains as a concept which is clearly valid in all groups that reproduce by sexual reproduction. Occasionally it has been suggested that species are artificial units created by taxonomists for their own convenience out of a mass of intergrading forms. While this may be so in parthenogenetic groups it is quite untrue of any group of sexual organisms that has been adequately studied.

Essentially, the species is a *breeding unit*—a group of individuals all of which normally and regularly breed together, except in so far as they may be separated by geographical barriers. Closely allied species may frequently be crossed under laboratory conditions, and may occasionally hybridize in the wild—but they do not do so normally, and their offspring are frequently sterile. Thus each species is separated from its neighbours in the systematic hierarchy, not only by morphological differences, but also by a variety of *isolating mechanisms*. The study of speciation thus largely resolves itself into an investigation of the nature of these mechanisms and of the manner in which they arise.

With the exception of a small number of cytoplasmic characters in plants (and possibly also in animals, but there is no well-established case as yet) all evolutionary changes have arisen in the first place as changes in the chromosomes. These changes seem to be of two kinds —single-gene mutations and alterations in the sequence of genes (structural rearrangements of chromosome regions). Although it is sometimes difficult to distinguish very minute rearrangements (inversions, deletions, &c.) from true gene-mutations, it is probable that the distinction between the two is a genuine one. In *Drosophila*, at any rate, structural rearrangements frequently lead to phenotypic changes which can be

interpreted as position-effects (i.e. the function of a gene depends to some extent on its immediate surroundings and undergoes a change when the gene is transferred to a new position in the gene-sequence).[43]

The detailed analysis of speciation by cytological and genetical methods has only just begun. Already, however, one thing is clear—the mechanism of speciation has not been the same for all groups. There are, in fact, many methods of speciation, and even within one family or one genus several may have been at work, so that only an elaborate investigation can lay bare the processes underlying the differentiation of species in each particular group. It is thus becoming increasingly difficult to formulate any general 'laws' of evolution, and such 'laws' as have emerged from morphological and palaeontological studies should be regarded with suspicion.

The chief limiting factors in determining the mechanism of speciation in a group are probably the method of reproduction, the mode of sex-determination, the possibility of geographical isolation and the effective size of the populations into which the species is divided. Where meiosis and fertilization are absent (as they are in organisms which are obligatorily parthenogenetic or have some vegetative method of reproduction) species tend to disappear, being replaced by a mass of intergrading forms which present the taxonomist with a fundamentally insoluble problem. Where true species do seem to exist in such groups they may be relics from the time when sexual reproduction still occurred in the group.

In sexual organisms we may distinguish four main types of genetic system:

(1) Hermaphroditism with self- or cross-fertilization.

(2) Bisexuality combined with a chromosomal method of sex-determination.

(3) Haplo-diploidy, as in the Hymenoptera and some other groups (see p. 41).

(4) Complex heterozygosity, as in some of the Oenotheras and a few other plants (not known in animals).

Speciation in the Higher Plants

Although in some groups of higher plants, such as the Gymnosperms, polyploidy seems to have played a relatively minor rôle in evolution, there can be no doubt that in the majority of the families of Angiosperms auto- and allo-polyploidy have occurred again and again. If one makes a list of the 'haploid' numbers of the higher plants (i.e. the somatic numbers divided by two) one finds that there is an excess of even over odd numbers amounting to about 40 per cent (see Table VII). This figure of 40 per cent gives

TABLE VII

Frequency of different Haploid Numbers in Phanerogams [52]

Haploid No.	No. of Species	Haploid No.	No. of Species	Haploid No.	No. of Species.
3	5	21	64	40	5
4	42	22	25	41	1
5	27	23	8	42	6
6	134	24	80	45	8
7	236	25	3	46	1
8	332	26	20	48	4
9	170	27	31	50	3
10	126	28	24	51	1
11	70	29	4	52	1
12	391	30	11	55	1
13	30	31	3	56	2
14	125	32	25	57	1
15	27	33	3	60	2
16	153	34	3	65	1
17	48	35	3	72	1
18	58	36	19	100	2
19	22	38	5		
20	47	39	1		

Total of even numbers:	1,646
Total of odd numbers:	768
Altogether:	2,414

a lower limit to the number of polyploid species of angiosperms. *At least* 40 per cent of all angiosperm species are tetraploid, &c. There is no other reason why more even than odd numbers should exist, and a detailed investigation of the frequency of particular numbers bears out the general conclusion. One cannot set an upper limit to the extent to which polyploidy has occurred in plants, since aneuploids and other 'derived polyploids' are not included in the above minimum figure. Neither is it possible to estimate the extent to which *all* Angiosperms have a more or less remote polyploid ancestry.

It is difficult to judge the relative importance of auto- and allo-polyploidy. The distinction between them is, however, only a relative one; on the one hand allopolyploids between very closely related species will be almost indistinguishable from auto-polyploids, while, on the other hand, independent mutations in the two diploid sets of an autotetraploid will eventually give rise to a tetraploid with extensive differences between its two diploid chromosome sets. Thus in two different ways forms will arise which are in some ways intermediate between auto- and allo-polyploids in their genetical constitution.

Whatever their origin, all even-numbered polyploids (tetraploids, hexaploids, &c.) will, as a result of independent mutation in their multiple chromosome sets, tend to evolve towards a new condition of genetical diploidy, in which no gene will be represented more than twice in the somatic chromosome set. There can be no doubt that many plant species represent stages in this process. It is probable that many mutations which would be lethal or sub-lethal in a diploid may occur in a tetraploid without lowering the viability of the organism, since two of the four genes (and similarly four of the six in a hexaploid) are more or less superfluous, and can consequently mutate without vitally affecting some important process, as usually happens when the genes of a diploid mutate.

It seems fairly clear that a number of new Angiosperm genera have arisen as a result of hybridization between fairly widely separated species, followed by a doubling of the hybrid chromosome set, just as *Raphanobrassica* (allotetraploid Cabbage-Radish hybrid) and *Aegilotriticum* (allopolyploid Wheat-Rye hybrid) have arisen in experiments. It is at any rate certain that several well-marked species like *Galeopsis tetrahit*, *Spartina townsendii* and the American form of *Phleum pratense* have arisen by allopolyploidy.[133, 72, 58]

On the other hand, where several chromosome numbers which are multiples of the lowest one exist within a single species of plant (as in *Biscutella laevigata*) it is probable that they have arisen by autopolyploidy. In many plant genera with extensive ' polyploid series ' it is not possible at present to decide which kind of polyploidy has occurred. The American species of *Crepis* have the following chromosome numbers, 11, 22, 33, 44, 45, 55, 76, 77, 86, 88 ; clearly there are some aneuploids as well as ' straight ' polyploids. We have already referred to the extensive polyploid series in the genus *Rumex*, but here again it is quite uncertain whether auto- or allopolyploidy has been at work.

In some apomictic (parthenogenetic) species of plants polyploidy is rampant, the chromosome numbers being highly variable and quite irregular. Thus in the grass *Poa alpina* the following numbers occur : 28, 33, 34, 35, 36, 37, 38, 39, 41, 45, 49, 52, 64, 65, 66, 67, 72, 73 and 74. Here meiosis is not necessary for reproduction and chromosome combinations can occur which would be impossible in a sexually-reproducing species.

In considering the general significance of polyploidy in the evolution of the higher plants it must be admitted that its chief rôle seems to have been as a quick method of producing species. A high polyploid is in a sense an evolutionary *cul de sac* ; it cannot give rise to new forms through further polyploidy (since

there seems to be an upper limit to the number of chromosome sets). Moreover, most mutations in an octoploid or a decaploid will be so completely recessive that they will hardly be acted upon at all by natural selection. There can be no doubt that many polyploid species are highly successful and well-adapted plants; but the evolutionary potentialities of a diploid are likely to be greater, in the long run.

Origin of Complex Heterozygote Organisms

Hypericum punctatum [65] is the only species of its genus which is a complex heterozygote, all the other St. John's Worts being normal diploids; its evolutionary origin is thus extraordinarily interesting, but cannot be analysed. *Rhoeo discolor* is the only representative of its genus, so that here again we have no method of determining the origin of the complex-heterozygote mechanism. The genus Oenothera, on the other hand, contains a series of forms ranging from ordinary diploid species to organisms which form a ring of fourteen chromosomes at meiosis (see Chap. V).

If we take one of the diploid species of Oenothera which must be regarded as ancestral to those which form rings we can consider two pairs of chromosomes:

abcdefghi	and	*mnopqrst*
abcdefghi		*mnopqrst*

If a reciprocal exchange (mutual translocation) takes place between one member of each pair of chromosomes it will give rise to two new chromosomes: *abcpqrst* and *mnodefghi*. The four chromosomes:

abcdefghi	*mnopqrst*
abcpqrst	*mnodefghi*

will now form a ring at meiosis; but there will not be any 'median segment' in such a ring; on the other hand, if a second reciprocal interchange takes place

it will establish a median segment if it does not correspond in position with the first. The second interchange may result from normal crossing-over if the first one was interstitial instead of terminal. By a repetition of this process rings of 6, 8, 10 and higher numbers can arise. The ring-forming species of Oenothera produce trisomic gametes fairly often as a result of one chromosome in the ring going to the wrong pole at the first meiotic division. They also produce new types of gametes as a result of occasional chiasma-formation in the median segments. The 'mutants' of De Vries (on which he based his 'Mutation theory')—were not really due to genetical mutation, but to occasional cross-overs of this type.

Chromosomal Evolution in Bisexual Organisms

In animals (with the exception of the hermaphrodite groups such as the Platyhelminthes, Oligochaeta and Hirudinea [185] and a few parthenogenetic Crustacea, Lepidoptera and Coleoptera) polyploidy is entirely absent, and consequently cannot account for the origin of new species. Nevertheless the range of variation in chromosome number is almost as great in animals as in plants. It is therefore clear that methods exist whereby chromosome numbers can be altered in the course of evolution. Actual loss or gain of whole chromosomes would probably lead to inviability in most diploid animals, so that it is probable that evolutionary changes in chromosome number have taken place in more indirect ways. Most changes in chromosome number in animals probably depend on some type of structural rearrangement of chromosome parts. Thus it is possible for most of the genetically active material in one chromosome to be transferred to another by means of a translocation, leaving only a small inert region behind, which may subsequently be lost from the chromosome set. It used to be supposed [190] that two or more chromosomes could

simply fuse together to form a single one, and, alternatively, that one chromosome could break into a number of fragments, each one of which would in future behave as a separate chromosome. If this were so a study of chromosome numbers would be of little importance. We now know, however, that each chromosome contains a single centromere, an essential part which is also a self-perpetuating body.[136] Thus fusion of two chromosomes will give rise to a dicentric one which will tend to break at mitosis (see p. 29) while breakage of a chromosome will give rise to a fragment lacking a centromere altogether. Thus simple 'fusion' and 'fragmentation' are alike incapable of giving rise to new types of chromosomes that are capable of indefinite survival.

One type of structural rearrangement that has undoubtedly occurred repeatedly in the course of evolution is the *inversion*. In some species inversions are found quite commonly in wild individuals. Thus in the IIIrd chromosome of *Drosophila pseudo-obscura* no less than 21 different inversions are now known from wild populations. No one gene sequence can be regarded as 'typical' for this chromosome, although some are commoner or more widely distributed than others. This is an extreme case, but there can be no doubt that inversions are common in many species of *Drosophila*, as well as in other groups of animals such as *Chironomus* spp. and various species of grasshoppers.

Translocations, although they may originate as often as inversions, do not seem to establish themselves in wild populations with the same facility. The reason for this is probably that individuals which are heterozygous for a translocation nearly always produce a considerable percentage of inviable gametes with 'unbalanced' chromosome sets. Thus so long as the new types of chromosomes produced by the translocation are rarer than the original ones they will tend to be eliminated by natural selection. Occasion-

ally, however, a wild population of a particular species may become reduced to a very few individuals, perhaps even to a single gravid female. Under such circumstances it may happen that a translocation manages to become established in a particular locality. There can be no doubt that translocations have occurred in evolution; but there are only one or two well-authenticated instances of their having been found in the heterozygous state in wild populations.[186] In *Drosophila* where a vast amount of work has now been carried out on the cytology of wild individuals not a single such case is known.

It is convenient to make a distinction between ordinary translocations and those where the breakage-points in both the chromosomes involved are very near the centromeres. In the latter it is whole arms of chromosomes (rather than portions of arms) which are transposed. Thus if we consider two chromosomes ABC.DEF and GHI.JKL (the point indicating the centromere) a whole arm transfer may give rise to two new types of chromosomes ABC.JKL and DEF.GHI. A translocation of this type probably gave rise to the X_1X_2Y sex-chromosome mechanism which is found in one group of Praying Mantids (cf. *Frontispiece*, Fig. 1). Again, if we consider two chromosomes with quasi-terminal centromeres ABCDEF.*g* and HIJKLM.*n* (where *g* and *n* represent minute regions beyond the centromeres) a translocation may give rise to the new chromosomes ABCDEF.MLKJIH and *g.n*. If the latter chromosome is inert (as it very well may be, since it is in any case minute) it may be eliminated from the set and the result of the translocation will be the apparent fusion of two rod-shaped chromosomes to produce a V-shaped one.

This kind of transformation seems to have happened repeatedly in some groups of animals. Thus most species of Short-horned grasshoppers (family Acrididae) have 11 pairs of rod-shaped autosomes. The Mexican species *Aidemona azteca* has only 10 pairs, but one of

these is V-shaped and has presumably arisen by a 'centric fusion' between two originally rod-shaped elements. Similarly *Aleuas vitticollis* has 9 pairs, two of which are V-shaped,[155] while the members of the genera *Chorthippus*, *Stenobothrus*, *Chrysochraon*, *Gomphocerus* and some others have only 8 pairs of autosomes, three of which are V-shaped.

It is, of course, not quite certain that these V-shaped grasshopper chromosomes have arisen by the method outlined above. It is at least possible that they possess two centromeres situated very close together (one derived from each of the original rods); but the balance of probability would seem to be in favour of their only having one.

The Evolution of the Sex-Chromosome Mechanism

The simplest type of sex-chromosome mechanism is where the X and Y are indistinguishable under the microscope, the difference between them residing either in a single gene or at most in a very short region of one or both chromosomes. Sex mechanisms of this kind, which cannot be studied cytologically, probably exist in some of the more primitive Diptera (Nematocera) and in the lower Vertebrates (Fishes and Amphibia). Although several workers have made detailed studies on the salivary chromosomes of the Chironomiidae, no difference between an X and a Y has ever been detected, so that if a 'differential' segment exists it must be very short (perhaps a single band).

From this very primitive type of sex-chromosome mechanism the subsequent history of the system may be described in general terms as a progressive increase in the extent of the differential segments at the expense of the homologous 'pairing segments'. Thus the X and Y, originally alike, become gradually more and more unlike. By the time the *Drosophila*-stage is reached the pairing segment has been reduced

to a relatively minute region which is nevertheless essential to the proper functioning of the mechanism, since it ensures that the two chromosomes pass to opposite poles at the first meiotic division. It is only in groups such as the Heteroptera, where the segregation of the X and Y does not depend on their forming a bivalent, that a pairing segment is dispensed with.

The differential segment of the X is frequently, and that of the Y almost always, inert (we have already stated that 'inert' regions are not absolutely functionless—thus an inert differential segment in the X may still possess sex-determining powers, although it does not have genetic properties in the ordinary sense). In some organisms, however, the differential segment of the X is largely or entirely active, as it is in *Drosophila* and the Fowl. Such organisms will possess large numbers of 'sex-linked' genes, whereas in forms with an inactive differential segment in the X few or no sex-linked genes will be found.

In many organisms the Y is smaller than the X, and in some groups it has been lost altogether.

Translocations occurring between sex-chromosomes and autosomes in the course of evolution may change the whole character of the sex-determining mechanism. Thus the inclusion of a pair of what were originally autosomes in an XO mechanism may convert it into an XY one again.[184, 188] We have already seen how a translocation of a different type may give rise to an X_1X_2Y mechanism, but not all systems of multiple X's and Y's have arisen in this way: some of them probably originated by reduplication of the original sex-chromosomes or parts of them.

Chromosomal Evolution in the Genus *Drosophila*

Even the most closely allied species of *Drosophila* probably differ in respect of a great many genes—say a hundred. But in addition to this they also differ in gene-sequence. It has, of course, long been known [122, 123] that the metaphase chromosomes of

many *Drosophila*-species are visibly different. But it was not until the introduction of the salivary chromosome technique that it was realized what a vast number of structural rearrangements had taken place during the evolution of the species in this one genus. Where species can be crossed it is possible to study the pairing of the salivaries in the hybrids and to compare the banding-patterns of the two sets of chromosomes with the greatest accuracy. Thus *D. melanogaster* and *D. simulans* are morphologically very similar and their mitotic chromosomes are quite indistinguishable. But in the salivary nuclei of the hybrid between them no less than 25 structural rearrangements are present, having presumably established themselves in one species or the other since they diverged in the course of evolution. One of these rearrangements is a large inversion in the IIIrd chromosome, six others are minute inversions, and the others are short regions where the chromosomes of the two species are not homologous, but where it is at present not possible to determine what kind of change has taken place.[66]

Drosophila pseudo-obscura, which occurs on the West Coast of North America from Alaska to Guatemala, has split in the course of evolution into two forms which seem to be completely isolated breeding-units, so that they should properly be considered as species, although they have usually been regarded as races of a single species. The two forms have different distribution areas, but there is a broad region of overlap where they occur together. Hybrids between Race A and Race B may be obtained, but they are sterile. A study of the salivaries in these hybrids has shown that there are at least four inversions present. The situation is complicated, however, by the fact that in *D. pseudo-obscura* there are many intra-racial inversions, so that for some of the chromosomes no one sequence can be regarded as 'normal' in preference to the others. Inversions

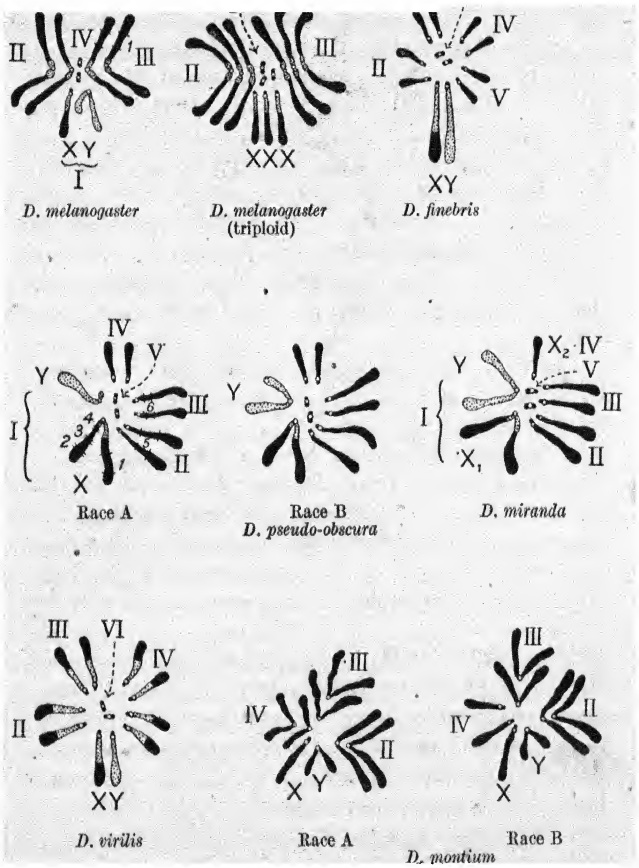


FIG. 20.—Somatic chromosome sets of various species of *Drosophila*; all male except the triploid *D. melanogaster*. Genetically active parts of chromosomes black, inert parts stippled. Inversion between *D. melanogaster* and *D. simulans* and between the A and B races of *D. pseudo-obscura* labelled 1, 2, 3, 4. . . . In *D. montium* the extent of the inert regions is not known so that they have not been indicated.

seem to be the only type of structural rearrangement that has managed to establish itself in the autosomes of *pseudo-obscura*, but at least seven different types of Y are known in the various strains of this species, all of which differ in size and shape. It is not possible to state exactly how these different types of Y have arisen, but probably both duplications and deletions have taken place in the evolutionary history of this chromosome. Since the Y is inert such increases and decreases of its substance will not seriously affect the genic balance of the organism. Nevertheless some of these types of Y lead to lethality in interracial hybrids.

The third member of the *pseudo-obscura* group is *D. miranda*, a rare and localized species recently discovered in certain localities in the Rocky Mountains.[42]. Morphologically it resembles *pseudo-obscura* very closely, and is evidently nearly allied to it. The mode of sex-determination in *miranda* is, however, unique in that it involves two pairs of chromosomes, the male being X_1X_2Y, the female $X_1X_1X_2X_2$. There is genetical as well as cytological evidence that the X_1 of *miranda* corresponds to the X of *pseudo-obscura*, while the X_2 corresponds to the IIIrd autosome. Thus in the evolution of *miranda* from a *pseudo-obscura*-like form one autosome has become haploid in the male sex, and is now associated with the sex-determining mechanism. Apparently what really happened was that the 'missing' chromosome or at any rate most of it became translocated to the Y. It was subsequently broken up into a number of much smaller portions as a result of structural rearrangements within the Y-chromosome. Thus the Y of *miranda* differs from those of other members of the genus in having a number of short active segments which represent the remains of the missing chromosome. Owing to the partial homology between X_2 and Y the former is associated with the X_1Y bivalent at meiosis and passes to the same pole as X_1 at the

first meiotic division. It is instructive to compare the X_1X_2Y mechanism of *Drosophila miranda* with the superficially similar one found in some Mantids. Both have arisen by the inclusion of a pair of autosomes in the sex-mechanism, but the former arose from an XY system, the latter from an XO one.

Drosophila pseudo-obscura and *miranda* can be induced to hybridize in the laboratory, but their offspring are entirely sterile. Very little pairing occurs in the salivary chromosomes of these hybrids, indicating that a large number of structural rearrangements have taken place since the evolutionary divergence of the parent forms. The vast majority of these rearrangements were undoubtedly inversions.

Drosophila melanogaster and *simulans* cannot be crossed with any member of the *pseudo-obscura* group, but a comparison of the salivary chromosome maps of these two groups of species reveals the fact that no regions of appreciable length have the same banding pattern. Thus the gene-sequence must have been completely changed and the chromosomes rebuilt by innumerable structural changes since the common origin of these two groups of species. It has nevertheless been shown by genetical methods that a general homology still exists between the chromosome limbs of *melanogaster* and *pseudo-obscura*—i.e. that the three rod-shaped pairs of autosomes of the latter contain approximately the same genes as the limbs known as IIL, IIR and IIIR in *melanogaster*, while the IIIL chromosome limb, which is autosomal in *melanogaster*, has become the 'right' limb of the X-chromosome in *pseudo-obscura*.

Thus, in spite of the vast number of structural rearrangements that have taken place, very few transferences of material from one limb to another have occurred; each chromosome arm is thus an entity which tends to retain its individuality throughout evolution, although the sequence of genes within it may be changed again and again by structural

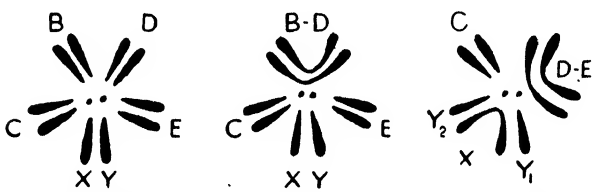
rearrangement. This is not to say that 'heterobrachial' rearrangements (i.e. ones involving more than one limb at a time) *never* occur: one or two undoubted cases are known to have happened in the phylogeny of the *Drosophila*-species. But the partial sterility that results from most types of translocations (whether between separate chromosomes or between the two limbs of a single one) is probably a very effective barrier to the establishment of translocations in natural populations.

The group of forms included under the general name of *Drosophila virilis* are a third example of a complex of incipient species within the genus.[67, 146] Three main species or subspecies may be distinguished: *D. v. virilis*, *D. v. americana* and *D. v. texana*. The former includes a number of different strains, some American, some Asiatic. A comparison between the mitotic chromosome sets of the three forms reveals some interesting differences. *D. v. virilis* has five pairs of rod-shaped chromosomes and a dot-chromosome. In *D. v. texana* two of these pairs are united to form a pair of V-shaped chromosomes. Genetical tests have shown that this fusion is between two autosomes that we may designate B and D. In *D. v. americana* two 'centric fusions' have taken place, but neither of these is the same as that which has occurred in *texana*; the first is between elements D and E, while the second is between element B and the X-chromosome. The effect of the latter fusion is to convert *americana* into an XY_1Y_2 form. That is to say, in addition to the original Y there is a 'neo-Y' which is not inert, and is in fact homologous to chromosome 'B' of *virilis virilis* and *virilis texana*. The three forms also differ in a number of inverted segments.

A detailed study of inversions in *Drosophila* populations has thrown a good deal of light on the evolution of categories lower than the species ('races', 'strains' and populations). Where a

number of different band-sequences are known for a particular chromosome it is often possible to construct genealogical trees of the various inversions. Since every structural rearrangement depends on at least two independent chromosome breaks (each of which is an event occurring only once in many million life-cycles) the chance of the same inversion arising more than once is negligible. It is to be hoped that eventually a detailed study of band-sequences will throw much light on the actual phylogeny of species in the genus *Drosophila*. Unfortunately this method can only be applied in forms possessing salivary chromosomes.

It was shown by Bridges that the salivaries of



D.v. virilis *D.v. texana* *D.v. americana*

FIG. 21.—Male chromosome sets of the three species or subspecies of the *Drosophila virilis* complex, showing the centric fusions that have occurred in *texana* and *americana* (after Patterson, Stone and Griffen, modified).

D. melanogaster contained a number of regions in which the banding-pattern was represented twice over. These regions, which he called *repeats*, are clearly homologous, in part at any rate, since they sometimes undergo pairing with one another. They are not confined to *melanogaster* but have been found in all those species of *Drosophila* that have been adequately studied.

Repeats can be interpreted as regions of the chromosome set that have been duplicated in the course of evolution, and are hence present in the tetraploid condition in an otherwise diploid species.

Some of them must be of considerable antiquity, since they are present in exactly the same position in *melanogaster* and *simulans*. The evolutionary significance of repeats is, as Bridges pointed out, that they 'offer a method of evolutionary increase in length of chromosomes with identical genes which could subsequently mutate separately and diversify their effects'. In other words, if we start with a repeat of the following type:

abcdefdefghi

independent mutation may change the chromsome to:

abcd'ef'de'fghi

(new allelomorphs being designated by apostrophized letters). Mutations which would be lethal in a gene which is only represented twice may be viable if they occur in one which is present four times. Thus repeats may possibly be an important kind of 'raw material' for future evolution.

It seems likely that inert (heterochromatic) regions have frequently undergone changes in length in the evolution of the genus *Drosophila*. In some species, such as *D. virilis*, the proximal heterochromatic segments are very long, whereas in others they are much shorter. Probably duplications and deficiencies of heterochromatic regions are particularly likely to establish themselves in the course of evolution, since they do not seriously upset the 'genic balance' of the organism.

Apart from the types of structural change already discussed, various other kinds of rearrangement of chromosomal material must have occurred in the genus *Drosophila*. Several groups of species have lost the small dot-like microchromosomes. Either this was the primitive condition or (more probably) in species such as *willistoni* and *saltans* the active

material of the dot-chromosome has been transferred to one of the other autosomes.

Lastly, in some species such as *ananassae* and the European *D. obscura* the number of chromosome-limbs is higher than in *melanogaster*, *virilis*, or *pseudo-obscura*, the rod-shaped chromosomes of the latter being represented by V's. Thus at some time in the past alterations in the positions of the centromere within the chromosome must have occurred.

Conclusions

In the seventy years following the publication of the *Origin of Species* the attention of biologists was directed mainly to the outward manifestations of the evolutionary process, rather than to a detailed analysis of the mechanism of evolution. The past fifteen years has witnessed a remarkable concentration of effort upon the latter aspect of the problem. Any final explanation of the processes of mutation and chromosome-breakage must be in terms of molecular biophysics; but from the standpoint of the evolutionary biologist it is possible to accept the existence of these two kinds of phenomena and proceed to analyse the evolution of particular species or groups of species by studying the distribution of different genes and gene-sequences in space or time. Such an analysis does not, of course, tell us much about the relationships of the main groups of the animal kingdom to one another—the type of problem that the biologists of the post-Darwinian generation were most interested in. It does, however, shed an entirely new light on the mechanisms underlying speciation. At last we are beginning to get a view of evolution 'from the inside'.

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